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EFFECT OF LOW-DOSE (1 KGY) GAMMA RADIATION AND SELECTED
PHOSPHATES ON THE MICROFLORA OF VACUUM-PACKAGED GROUND
PORK

Iowa State University

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Effect of low-dose (1 kGy) gamma radiation and selected
phosphates on the microflora of vacuum-packaged ground pork

by

Robson M. Ehioba

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major: Food Technology

Approved:

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DEDICATION

In memory of my late mother whose insight helped shape
my future endeavors.

INTRODUCTION

The use of low-dose gamma radiation to extend the shelf life of fresh meats has been proposed by several investigators (Anellis et al., 1967; 1969; and 1972; Niemand et al., 1981; Sivinsky, 1983). Low-dose gamma radiation may be used to eliminate pathogens, particularly Salmonella, in poultry and red meats. The use of this technology may be particularly important in poultry processing in view of the high contamination rates with Salmonella and other pathogens in poultry.

The possible occurrence of trichinosis in pork has limited U.S. pork exports. The National Pork Producers Council (NPPC) Committee on Trichina-Safe Pork had recommended that the NPPC pursue a policy to provide a nationwide supply of trichina-safe pork by January 1, 1987 (Sivinsky, 1983). A promising method for rendering pork non-infectious in relation to trichina is gamma irradiation, one of the approaches identified by the NPPC to accomplish that goal. There are some European countries that do not allow the importation of United States pork because there is no guarantee that it is trichina-free. Other countries require that pork be certified as non-infectious for trichina before it can be allowed in their markets. Pork producers believe that developing trichina-free pork through

irradiation processing would do much to bolster the United States sagging pork export market. Low-dose gamma irradiation (30-100 krad) processing of pork, which was approved by the Food and Drug Administration effective July 1985 (FDA, 1985), might also help in reducing the number of microorganisms that are responsible for spoilage of fresh meats.

The application of low-dose gamma radiation has potential for inhibiting sprouting of tubers and root vegetables, inhibiting postharvest growth of asparagus and mushrooms, insect disinfestation, delaying ripening and senescence in fruits and controlling post harvest diseases in fruits and vegetables. The possible use of ionizing radiation for insect disinfestation is one of this technology's most promising application in fruits and vegetable processing.

In view of the numerous advantages associated with the use of ionizing radiation in food processing, it is surprising that the use of this technology is so hesitantly accepted by consumers and some food processors.

The objective of this study was to determine the effect of low-dose (100 krad) gamma radiation and selected phosphates on the microflora of vacuum-packaged ground pork.

Microbiology of Fresh Meat Spoilage

Fresh meat is very susceptible to microbial spoilage because of the abundant availability of nutrients capable of supporting microbial growth. The rapid spoilage of fresh meats, which is mainly due to psychrotrophic Pseudomonas spp. has long been a problem to the meat industry. Several investigators have reported on the use of vacuum-packaging to extend the shelf life of fresh meats beyond the traditional storage period of five days (Clark and Lentz, 1972; Huffman, 1974; Silliker et al., 1977; Sutherland et al., 1977; Christopher et al., 1979; Enfors et al., 1979). It has been claimed that high oxygen concentration can prolong the red color of meat (Taylor and Macdougall, 1973, Taylor, 1977). Butler et al. (1953) in a classical report, stated that there is a relationship between bacterial growth, meat discoloration and slime production and that, as bacterial growth increases, there is a corresponding increase in meat discoloration and slime production. Johnson (1974) reported that since meat tissue and bacteria present on it utilize oxygen, the oxygen still available after vacuum-packaging the meat decreases with time, while the concentration of carbon dioxide increases. However, on exposure of the vacuum-packaged meat to oxygen, it regains its desirable bright red color.

Simard et al. (1983) determined the effects of temperature, light and storage time on the shelf life of vacuum- and nitrogen-packaged sausage. It was found that total numbers of lactobacilli, psychrotrophic and anaerobic bacteria were not statistically different during storage. However, Lactobacillus counts were higher in nitrogen gas than in vacuum-packaged samples. The initial predominant flora was found to be constituted by Pseudomonas and Microbacterium but, as storage time increased, the Lactobacillus became more predominant. In a similar study conducted by Finne (1982), modified and controlled atmosphere storage of muscle foods were studied. The author found that vacuum packaging had the potential to reduce growth of common aerobic spoilage bacteria.

Huffman et al. (1975) studied the effect of gas atmospheres on microbial growth, color and pH of ground beef. In their findings, it was reported that modified gas atmospheres were preferred over vacuum packaging as a means of reducing microbial spoilage of ground beef. In an earlier study conducted to determine bacterial growth in refrigerated, vacuum-packaged luncheon meats, Kempton and Bobier (1970) found that CO₂ and N₂, when used in controlled atmospheres, extended shelf life better than vacuum-packaging for refrigerated luncheon meat.

There are controversial reports in the literature

regarding the growth of Pseudomonas in vacuum-packaged meats. Sutherland et al. (1975) reported such growth. Seideman et al. (1976) also found Pseudomonas sp. in vacuum-packaged fresh meats. However, in studies by Pierson et al. (1970) and Roth and Clark (1972), the growth of Pseudomonas in vacuum-packaged fresh meats was not observed.

In a comprehensive review by Eustace (1981), it was stated that vacuum-packaged meats have longer shelf life because of the inability of aerobic meat spoilage microorganisms to grow. It was further reported by the author that keeping bacterial contamination to a minimum can contribute to shelf-life extension. Gradual depletion of oxygen and an increase in carbon dioxide concentration have been credited to a reduction in numbers of aerobic spoilage microorganisms in vacuum-packaged meats, thereby increasing the shelf life of the product (Newton and Rigg, 1979).

Egan and Shay (1982), studied the spoilage of vacuum-packaged beef stored at 5°C. They found that in the absence of detectable spoilage organisms, the vacuum-packaged product spoiled due to off flavor development as evaluated by analytical taste panels. The rate of spoilage was reported to increase with a corresponding increase in film permeability. In a similar study conducted by Egan et al. (1980), the rate of spoilage of vacuum-packaged, sliced luncheon meats stored at 5°C brought about by Brochothrix

thermosphacta was higher than that of the lactobacilli. Further results from the study revealed that product spoilage was rapid when the microbial population of the product approached 10^8 cells/cm². Earlier, Patterson and Murray (1975) had studied the development of the microbial flora of vacuum-packaged beef stored at 5°C. The authors observed that as storage proceeded, the proportion of lactic acid bacteria on the meat increased over that of aerobic organisms. However, the numbers of aerobic spoilage microorganisms continued to increase throughout the storage period.

Tandler and Heinz (1970a, b; 1971) reported that wraps relatively permeable to oxygen supported meat spoilage more than oxygen impermeable wraps because the concentration of CO₂ inside the impermeable wraps contributed to inhibit growth of aerobic spoilage microorganisms. However, higher temperatures and longer storage time were shown to increase bacterial counts in vacuum-packaged beef stored at 0 and 4.4°C. Vanderzant et al. (1982) studied the effect of packaging beef loin steaks with different oxygen barrier films. They discovered that lactic acid bacteria dominated in vacuum-packaged fresh beef when stored at refrigerated temperatures. Lactobacillus spp. were reported to account for more than 50% of the microflora of all samples that were stored at 2 to 7°C. Microorganisms found to comprise the

the other 50% were species of Leuconostoc, Brochothrix, Aeromonas, Pseudomonas, and Streptococcus. The initial microflora composition, time and temperature of storage and gas permeability characteristics were suggested to determine the type of microorganisms found in refrigerated, vacuum-packaged ground beef.

Various reports on the microbiology of vacuum-packaged meats have shown that lactic acid bacteria are responsible for the production of acid flavors, sour odors and gas (Patterson and Gibbs, 1977; Hanna et al., 1979; and Savell et al., 1981). The importance of Leuconostoc spp. in vacuum-packaged meats in a processing plant was determined in a study of sensory and microbial profiles of steaks packaged in O₂-CO₂-N₂ atmospheres by Savell et al. (1981). It was found that Leuconostoc spp. dominated the microflora of the meat when stored in O₂-CO₂-N₂ atmospheres. After 14 days, 'sour' or 'cheesy' odors were detected.

Chandran et al. (1986) studied the relationship between slaughter-dressing, fabrication and storage conditions and the microflora and sensory characteristics of vacuum-packaged beef steaks. A direct relationship between strict sanitary fabrication practices and a reduction in total microbial numbers was found. The same direct relationship existed between sanitation and incidence of gram-negative spoilage microorganisms associated with fresh

meat spoilage under conditions of oxygen availability. Baltzer (1969) showed that lactic acid bacteria, which become dominant in vacuum-packaged meats, do not cause as much damage to meat quality as the aerobic spoilage microorganisms do. As a result, several investigators have suggested the use of vacuum packaging as an effective means to control microbial spoilage of fresh meats (Collins-Thompson and Lopez, 1980; Hodges et al., 1974).

Sources of Gamma Radiation for Food Preservation

The unit of radiation energy absorbed by matter is the rad (radiation absorbed dose). One rad is defined as the absorption of 100 ergs of energy by one gram of matter being irradiated. The use of the rad as a unit of radiation absorbed dose is being gradually replaced by the Gray (Gy), which is defined as the absorption of 1 Joule (J) of energy by each Kilogram of matter being irradiated. A kilorad of radiation is equal to 1000 rad while the Gy is equal to 100 rad, and 1000 Gy is equal to 1 kGy (kiloGray). An electron Volt (eV) is defined as energy gained by an electron when accelerated by a potential of 1 Volt. One million electron Volts (eV) is designated by MeV. A Curie (Ci) is a unit of radioactivity that results in the disintegration of 3.7×10^{10} atomic nuclei/sec.

There are two different types of radiation sources utilized in the preservation of foods. These are electron beam accelerators and the isotopic sources cobalt-60 (^{60}Co) and cesium-137 (^{137}Cs). Electron beam radiation is produced by a machine where a beam of fast moving electrons in a vacuum bombards a metallic target. X-ray is an example of electron beam radiation. In isotopic sources of radiation, spontaneous disintegration of the atomic nucleus of radioactive compounds (^{60}Co and ^{137}Cs) produce electromagnetic radiations of short wavelength, i.e., gamma rays.

The decision as to which of those two types of radiation sources to use in food irradiation depends on the physical characteristics of the radiation source (as it relates to radiation penetration energy), economics of the process, product characteristics, isotope availability, throughput needs and dose rate.

The possible formation of induced radioactivity is an important issue to consider when deciding on what radiation source to utilize for food irradiation. Induced radioactivity has been shown to be closely associated with electron sources of radiation due to their high energy emission capacity, whereas the commonly used radioisotopes (^{60}Co and ^{137}Cs) do not emit high enough radiation energy to induce radioactivity (FAO/IAEA/WHO, 1981). However, the

same organization reported that induced radioactivity from the use of electron accelerators is minimal and short-lived if the applied energy level is below 16 MeV. As a result, the Committee on the Wholesomeness of Irradiated Foods (FAO/IAEA/WHO, 1981) recommended that the "radiation permitted for food irradiation" in the case of electron sources be 10 MeV and 5 MeV for gamma rays and X-rays, respectively.

Although the use of radionuclide sources in food preservation has been shown to be safe and efficient (FAO/IAEA/WHO, 1977), there are some limitations to their use in food processing at present. The supply of both ^{137}Cs and ^{60}Co is limited, so that isotope production would have to be increased to support food irradiation needs as the use of the technology expands worldwide. The United States government, which has discontinued the recovery and purification of ^{137}Cs from nuclear waste, has only about 77 million curies (MCi) in supply, while ^{60}Co , produced almost totally by a Canadian government agency at a rate of about 20-25 MCi/year, could not meet the needs of the food industry if food irradiation were to be applied in large scale (FAO/IAEA/WHO, 1981) without adding new reactor-producing capacity. The U.S., however, would be able to enter the production of ^{60}Co if needed. Although irradiation of foods can be carried out at low (<100 krad),

medium (100-1000 krad), or high doses (>1000 krad), the geometry, density, size and other characteristics of the product have to be considered in order to provide the needed doses to control microorganisms present in foods.

As for electron beam accelerators, their characteristics of low penetration restrict their use almost totally to the treatment of non-food products (i.e., plastics) whereas the isotopic sources are ideally suited to food processing applications (WHO, 1966).

An important feature of the electron source is that it is possible to construct portable systems capable of handling high product volume. One other advantage associated with electron beam accelerators is the directionality of the electron beam. Electron beams can be directed, thereby allowing for a more efficient use of available beam energy, whereas the isotopic sources are multidirectional. Further, the electron beam sources can be turned off and on when needed, something not possible with isotopic sources.

Therefore, in deciding which type of radiation source to use, a strong consideration should be given to the probable advantages and disadvantages associated with either radiation source. However, the isotopic sources do have a competitive edge over electron sources because of their high product penetration capabilities.

Methodology of Food Irradiation

At a food irradiation facility, packaged food is loaded on conveyors, and gamma rays pass through the food in a shielded chamber at controlled doses. Cobalt 60 or Cesium 137 capsules are raised from a pool of water thereby allowing the food to absorb a predetermined radiation dose that is regulated by time of exposure and measured by means of dosimeters. The shielded chamber prevents gamma rays from escaping. The processed food does not become radioactive.

Radicidation is the treatment of foods with ionizing radiation at doses that destroy pathogenic bacterial cells (2-5 kGy). Radicidation results in food products similar to those that have been pasteurized by heat, and as pasteurized foods, refrigeration is required for foods pasteurized with gamma radiation. The main advantages of radicidation are its potential to produce pathogen-free products and to extend shelf-life of the treated food. This process can be used to delay spoilage and reduce pathogens in red meats and poultry.

Radappertization is a radiation sterilization process whereby food is exposed to high levels (2-5 Mrad) of gamma rays. In resemblance to heat-sterilized foods, radappertized foods can be stored in cans or pouches for

several years without refrigeration so long as the food package is not damaged. This is particularly applicable in developing countries where refrigeration is sometimes unavailable and conventional food preservation techniques are expensive.

Preservation of Meats by Use of Low-Dose Gamma Radiation

It has been demonstrated that irradiation can reduce the microbial load of fresh meats, thereby increasing the useful life of the perishable meat product (Niemand et al., 1981; Sivinsky and Switzer, 1983; Mattison et al., 1986).

At medium doses (100-1000 krad), irradiation may also be used to eliminate pathogenic bacteria from meats, directly through "cold pasteurization" due to irradiation, or indirectly by destroying pathogens in animal feeds. Salmonella in poultry has been shown to be derived from feed and other sources. Since the incidence of salmonellae can be reduced through irradiation of feeds (FAO/IAEA/WHO, 1977), that process may contribute a means of controlling Salmonella in poultry and eggs. Licciardello et al. (1969) reported on the possible inactivation of Salmonella by treating poultry with gamma radiation. The authors showed that Salmonella oranienburg was the most radiation resistant, whereas S. newport was the most radiation

sensitive of all the serotypes studied. Other investigators (Comer et al., 1963; Ley et al., 1963; Mossel and deGroot, 1965; Quinn et al., 1967) have also reported that there is a variation in radiation resistance among salmonellae serotypes. Besides Salmonella control, reports by Proctor et al. (1956), McGill et al. (1959) and Coleby et al. (1960) have shown that the shelf life of poultry stored at above-freezing temperatures can be extended by use of ionizing radiation. Coleby et al. (1961) also determined that it was important to maintain low temperatures during radiation processing of meat in order to retard the radiation-induced off-flavors and odors that have been reported to be produced during irradiation at ambient temperatures. Grecz et al. (1965) stated that beef briskets frozen with liquid nitrogen retained their organoleptic quality when irradiated at -196°C .

The on-shore irradiation of fish and seafood has received much attention because of its potential to control the incidence of Vibrio parahaemolyticus, one of the most important seafood borne disease agents in warmer climates (Giese, 1976).

Most of the research conducted on the use of low-dose irradiation for the preservation of fresh meats is limited to fresh beef. Anellis et al. (1975), in a study on the effect of low-temperature irradiation (2.6 Mrad) of canned

beef inoculated with spores of Clostridium botulinum types A and B, found that a few of the inoculated cans were positive for Clostridium botulinum type B toxin, but did not contain type B viable cells. It was also discovered that other cans had both types A and B toxins. The results showed that bacterial toxins were more resistant to radiation than vegetative cells. Grecz et al. (1965) studied the effect of temperature on the radiation resistance of spores of Clostridium botulinum. They found that, as the radiation temperatures decreased, there was a corresponding increase in the radiation resistance of C. botulinum spores. In a separate study involving precooked ground beef seeded with C. botulinum 33A spores at a concentration of 2.3×10^8 /can and irradiated (3.6-3.9 Mrad) under temperatures in the range of -195 to 95°C, a linear increase in clostridial spore radiation resistance with a decrease in temperature was found (Grecz et al., 1965). Wheaton et al. (1961) studied the radioresistance of five strains of Clostridium botulinum in selected food products and found that strain 12885A was most resistant to radiation. Numbers of 12885A viable spores decreased from 5.8×10^6 /g to 8.5×10^3 /g with a radiation dose of 2.5 Mrad. Sinnhuber and Landers (1964) irradiated codfish inoculated with C. botulinum spores at 4.5 Mrad at room temperature and found no incidence of Clostridium botulinum. Clifford and Anellis (1975)

irradiated 8 strains of Clostridium perfringens spores at -30°C in Sorensen phosphate buffer and found that all eight strains probably (95% level) followed a non-exponential rate of death. Niemand et al. (1981) studied the shelf life of vacuum-packaged, irradiated prime beef cuts. They discovered that irradiating beef at 2 kGY resulted in a reduction of Pseudomonas, Enterobacteriaceae and enterococci. It was further reported by the investigators that lactic-acid producing microorganisms were resistant to a medium-dose (2 kGY) irradiation, so that an increase in the number of lactic acid bacteria toward the 11th day of storage (4°C) was observed.

Keller and Maxcy (1984) studied the physiological age-dependent variation in radioresistance for three radiation resistant bacteria: Micrococcus radiodurans, Micrococcus sp. isolate C-3 and Moraxella sp. isolate 4. They found that M. radiodurans and isolate C-3 were more resistant to gamma radiation when the stationary phase of growth had been reached than during logarithmic growth. In the case of Moraxella sp. isolate 4, the radiation resistance was found to be the reverse of the other two cultures. Other investigators (Stapleton, 1955; Bridges and Horne, 1959; Morton and Haynes, 1969) had also observed differences in the radiation resistance of Micrococcus radiodurans, E. coli and Salmonella typhimurium and reported M. radiodurans as

most radiation resistant. Welch and Maxcy (1975) reported on the characterization of radiation resistant bacteria in ground beef and found most of the isolates to be Moraxella after irradiating the ground beef at 200 krad.

Radiation-Induced Shift in Microflora of Fresh Meats

Several studies have been conducted on the use of low-dose gamma radiation to control the microflora of fresh meats (Anderson et al., 1956; Heiligman, 1965; Welch and Maxcy, 1975; Rowley and Brynjolfsson, 1980; Niemand et al., 1981; and Keller and Maxcy, 1984). Numerous reviews have been published that tend to support a radiation-induced microfloral shift upon low-dose gamma irradiation of meat (Tiwari and Maxcy, 1971; Anellis et al., 1973; 1975; Dickson and Maxcy, 1985). Tiwari and Maxcy (1971) studied the impact of low doses of gamma radiation and storage conditions on the microflora of ground beef. It was discovered that meat samples irradiated at 68 krad had only 0.2% of the number of microorganisms found in the unirradiated samples. They further reported that while the fresh, unirradiated samples contained mostly gram-negative, nonsporeforming microorganisms, the irradiated counterpart contained mostly gram-positive, nonsporeforming rods when stored at 5°C. Ingram and Farkas (1977) observed that the

microflora present in meat before and after irradiation differed greatly due to a shift from mostly gram-negative to gram-positive microorganisms. In a report by Wolin et al. (1957), it was stated that lactic acid bacteria were the predominant spoilage microorganisms in irradiated (200 krad), vacuum-packaged fresh meat stored at refrigeration temperatures, while gram-negative microorganisms (i.e., Pseudomonas) predominated in unirradiated fresh meat. Urbain (1983) found that the gram-positive lactobacilli dominated the microflora of irradiated (1-2 kGy), vacuum-packaged meat samples stored at 4°C. Pelroy and Eklund (1966), working with irradiated (200 krad) fish, and Niemand et al. (1981), who worked on the microbiology of irradiated beef, reported similar shifts in microflora from mostly gram-negative to gram-positive microorganisms due to medium-dose (2 kGy) gamma irradiation. Smith and Palumbo (1983) suggested that irradiation may be used to treat meat for fermented sausage production, a process that relies on the dominance of lactic acid bacteria in the microflora to produce the desired flavor and inhibit the growth of pathogens. In a study conducted by Tiwari and Maxcy (1971) on the impact of low doses of gamma radiation and storage at 5°C on the microflora of ground red meat, it was found that an irradiation dose of 68 krad reduced numbers of gram-negative microorganisms. The irradiated samples were

reported to contain fewer microorganisms than the unirradiated samples. The evidence showed the efficacy of low-dose irradiation in reducing the numbers of bacteria that cause fresh meat spoilage.

Several studies are available that show that radiation-sensitive bacteria dominate the microflora of unirradiated and low-dose irradiated foods (Proctor and Goldblith, 1951; Tarpley et al., 1953; Hannan, 1956; Koh et al., 1956; Rayman and Byrn, 1957; Niven, 1958; Erdman et al., 1961; Thornley, 1963; and Christensen and Holm, 1964). In contrast, Anderson et al. (1956), who studied the radio-resistant Micrococcus in ground beef, found that Micrococcus radiodurans, a nonspore former, showed a high degree of radiation resistance.

In a report presented by the World Health Organization (FAO/IAEA/WHO, 1977) on possible radiation-induced genetic variations, it was stated that only in laboratory animals has that risk been confirmed. The report also stated that, other than the laboratory results, there were no findings to rationalize earlier concerns about the evolvement of radiation-induced mutation of microorganisms in foods (WHO, 1981). Aravindakshan (1975) found that there were no observable radiation-induced changes in pertinent taxonomic characteristics of the microorganisms studied that could be attributed to mutation. According to FAO/IAEA/WHO (1981),

there were no documented studies demonstrating increased radiation-induced pathogenicity of food-borne bacteria. Therefore, those agencies continue to hold the view that irradiation processing of food does not in any way increase the pathogenicity of microorganisms. In laboratory studies that were conducted under conditions different from those occurring in practice, it was found that mycotoxin production by molds originating from irradiated spores was different from that of the unirradiated parent strain (WHO, 1970). An earlier report (WHO, 1966) stated that increased mycotoxin production by molds had been reported by investigators who used heavy mold inocula in irradiated, autoclaved foods.

The possible survival of radiation-resistant microorganisms in foods is of significant concern, but studies conducted on that possibility have shown that no new health hazard associated with such organisms exist (WHO, 1970). Reports by the FAO/IAEA/WHO (1981) showed that irradiation, when used in combination with heat and/or salt treatments, have an increased efficacy to limit the growth of highly radiation-resistant microorganisms. Several other studies have been conducted on food irradiation using Clostridium botulinum spores (which are highly radiation resistant), as safety indicator organisms. Denny et al. (1958) studied the destruction of C. botulinum by ionizing

radiation using spores from five type A and type B strains, at a concentration of 1×10^6 spores/can in parboiled ground pork at -20°C . They obtained a D value of about 0.35 Mrad. Grecz et al. (1965) conducted a study on the radiation resistance of spores of C. botulinum at 0 and -196°C in phosphate buffer and in ground beef. They discovered that rapid freezing with liquid nitrogen plus irradiation, decreased the viability of C. botulinum spores in phosphate buffer. However, irradiation of inoculated ground beef at a higher temperature and at doses of 2.5 to 3.5 Mrad was reported to result in 100% spoilage, while meat irradiated at 3.0 to 3.6 Mrad only supported partial spoilage. The findings also revealed that 3.9 Mrad prevented swelling of the cans.

History and Regulatory Aspects of Food Irradiation

The first studies on food irradiation in the U.S. were conducted in the early 1950s, sponsored mainly by the U.S. Atomic Energy Commission (AEC) and the U.S. Department of the Army (DA). Food irradiation, as a process, experienced two important setbacks in the 1960s: the FDA's denial of a DA petition for re-irradiation of irradiated ham and, in agreement with the 1958 Amendment to the Federal Food, Drug and Cosmetic Act (FD&C), the FDA reversal of the

authorization for irradiation of bacon. The legislation passed redefined food irradiation as a food additive rather than a process, so that the FDA was thereafter required to approve its use in foods. Newer legislation also required that the packaging materials that come in direct contact with the food during radiation processing must receive the FDA's approval (U.S. Code of Federal Regulations, 1984). In a report released by the FAO/IAEA/WHO (1977) in conjunction with the Joint Expert Committee on the Wholesomeness of Irradiated Foods (JECFI), the available research results on the wholesomeness of nine irradiated foods were reviewed. The conclusions reached were "1) recognition of food irradiation as a process (controverting the 1958 FD&C Act); 2) recognition of the importance of the radiation chemistry approach to wholesomeness evaluation, in tandem with animal feeding and cytotoxicity studies; 3) unconditional acceptance of irradiation of wheat and wheat products (15-100 krad for sprout inhibition), chicken (200-700 krad for refrigerated shelf life extension and pathogen elimination), papaya (50-100 krad for prolongation of fresh market life by partial elimination of spoilage organisms); and 4) provisional (pending unconditional) acceptance of irradiation of onions (2-15 krad for sprout inhibition), fresh cod, and red fish (100-200 krad to reduce numbers of spoilage and pathogenic microorganisms and to extend

refrigerated shelf life at or below 3°C), and rice (10-100 krad for insect disinfestation)".

Two publications: General Standards for Irradiated Foods, and A Code of Practice for the Operation of Irradiation Facilities Used in Treatment of Foods were presented for general review by the Codex Alimentarius Commission (CAC) in 1977. The publications contained the decisions and suggestions of a JECFI meeting held in 1976 and became the standard and code of practice at their 1979 meeting. However, the FDA, although represented at JECFI and CAC, developed its own procedure for ensuring that irradiated foods were safe based on a review of toxicological data on the safety of irradiated foods (Giddings and Welt, 1982).

USDA's Food Safety and Inspection Service is the government agency that has the responsibility for enforcing the safety and wholesomeness of meat and poultry products marketed in the United States, so any potential use of radiation for processing of meat and poultry must be approved by the FSIS. An amendment to the Federal Meat Inspection Regulations was adopted in January, 1986 by the FSIS to allow for the irradiation of fresh or previously frozen pork at 30-100 krad for the purpose of trichina inactivation, a dose range that had previously been approved for the processing of fresh pork by the FDA (AIII, 1987).

The FDA also issued a final rule in April, 1986 that allowed food processors to apply radiation doses of up to 100 krad to disinfest fruits and vegetables, and to delay their maturation. Also, the use of radiation doses between 1000 and 3000 krad to eliminate microorganisms in spices and dried vegetable seasonings was permitted by the aforementioned rule. In the area of labeling of irradiated foods, the FDA required that all foods treated by ionizing radiation bear a logo, and a statement "Treated with radiation" or "Treated by irradiation" (Newsome, 1987).

At present, there is no commercially available method for detecting whether a particular food has been irradiated or not and at what dose. For that reason, the FSIS, in conjunction with the National Bureau of Standards (NBS), are developing a post-irradiation dosimetry method that would determine whether or not pork and poultry had been previously irradiated (AIII, 1987).

At this writing, there are six irradiation petitions, dealing with different irradiation issues, waiting to be approved by the FDA (Food Chemical News, 1985). The Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture has acknowledged the significance of irradiation for the reduction of pathogens in meats and poultry, and for controlling helminths in pork. Therefore, the next several years could show a spiralling upward growth

in the application of this technology to food processing.

Antimicrobial Properties of Phosphates in Fresh Meats

The available information on the antimicrobial properties of phosphates in fresh meats is limited. However, extensive research has been done on the use of phosphates in cured meats to enhance flavor, water retention, color, tenderness and juiciness and to decrease cooking shrinkage (Swift and Ellis, 1956; 1957; Klose et al., 1963).

Molins et al. (1985) studied the effects of phosphates on aerobic mesophilic and psychrotrophic bacterial growth, and on survival of inoculated Staphylococcus aureus Z88 in refrigerated uncooked bratwurst stored at 5°C for 7 days. The authors reported that, although 0.5% sodium acid pyrophosphate (SAPP) was effective in lowering total aerobic mesophilic and psychrotrophic counts, sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP), and sodium polyphosphate glassy (SPG) did not contribute significant reductions in microbial numbers. Furthermore, none of the phosphates used in the study had a significant ($P < 0.05$) effect on the survival of the inoculated S. aureus.

Earlier, Molins et al. (1984) studied the recovery of selected bacteria in laboratory media containing 0.5% food

grade poly- and pyrophosphates using pure cultures of Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus and two lactic starter cultures. It was discovered that tetrasodium pyrophosphate (TSPP) was the most inhibitory of all phosphates tested, while heated sodium tripolyphosphate (STPP) and sodium polyphosphate glassy (SPG) were found to be less inhibitory. However, sodium acid pyrophosphate (SAPP) neither inhibited or increased the ability of the microorganisms to recover in the phosphate media.

Fristenberg-Eden et al. (1984) reported that sodium phosphates exert varied effects in preventing colony formation in unstressed and heat-stressed Moraxella-Acinetobacter. Of all phosphates (sodium tripolyphosphate, sodium pyrophosphate and sodium orthophosphate) tested on unstressed Moraxella-Acinetobacter, 0.1% sodium tripolyphosphate was found to be the most inhibitory, while sodium orthophosphate was the least inhibitory to the microorganisms tested. It was further reported that the inhibition of colony formation by sodium tripolyphosphate was unrelated to pH.

The inhibition of Clostridium botulinum vegetative cell growth by 0.5% sodium tripolyphosphate in media containing 1.5% potassium sorbate was reported by Seward et al. (1982). It was discovered that phosphate-treated Clostridium

botulinum cells were abnormal in shape and cell division due to the action of phosphate. In a similar study conducted by Wagner and Busta (1984) to determine the interactions of pH with sodium acid pyrophosphate (SAPP) in media against Clostridium botulinum, it was found that growth of different strains of the organism was delayed by SAPP and a synergistic action between SAPP and potassium sorbate was observed. Other reports are available on the synergistic relationship between pH and phosphate against bacteria in fresh and processed meats (Roberts et al., 1981a; b; Nelson et al., 1983; Wagner and Busta, 1983; Sofos, 1985). In a study conducted to determine the effect of 0.3% Curaphos 700 (a commercial polyphosphate blend) added to a "low" pH (5.5-6.3) pork slurry, Roberts et al. (1981a) found that phosphate increased the probability of toxin production by Clostridium botulinum. The opposite took place in "high" pH (6.3-6.8) pork slurry (Roberts et al., 1981b). Madril and Sofos (1985) showed that antimicrobial effects observed in meat preparations containing sodium acid pyrophosphate (SAPP) were due to lower pH and phosphate ion present in the meat formulation. The authors also concluded that the antimicrobial effect of SAPP was greater at pH 6.0 than at pH 5.7 or 6.3. Jarvis et al. (1979) discovered that the effectiveness of diphosphates against Clostridium botulinum growth and toxin production was greater than that of

tripolyphosphates. Sofos (1985) reported that at higher NaCl concentrations, there was a tendency for sodium tripolyphosphate to show higher antimicrobial properties.

Commercial mixtures of phosphates have been shown to inhibit the growth of bacteria in meat products. Spencer and Smith (1962) found that dipping poultry meat in a polyphosphate solution increased the shelf life of chicken by about two days. Post et al. (1963) also described the antibacterial properties of sodium hexametaphosphate used to dissolve calcium alginate swabs. They reported that polyphosphates are strong chelating or sequestering agents for metallic ions. Klose et al. (1963) theorized that the antimicrobial activity of straight-chain phosphates is due to their complexing, dispersing, stabilizing and peptizing actions. Chelating agents may either increase or reduce growth of microorganisms. Metals essential for growth have been shown to be more available after chelation by absorbable compounds (Lankford et al., 1957). Weinberg (1957) reported chelation of metal ions to affect the efficiency of antibiotics, and Jay et al. (1957) suggested that chlortetracycline may owe its antimicrobial action in beef to chelation of trace metals required by microorganisms.

Elliot et al. (1964) studied the inhibitory effects of polyphosphates on pseudomonads isolated from poultry meat.

The authors found that the use of 3 and 8% phosphate blend (Kena) in a broth medium totally eliminated the nonfluorescent pseudomonads, while the fluorescent strains were temporarily affected but grew after a prolonged lag period. The aforementioned inhibition was attributed to the chelating properties of the polyphosphates on ions needed by the microorganisms for survival. Chen et al. (1973) studied the effects of water and microwave energy precooking on the microbiological quality of chicken parts. They reported that gram-positive Micrococci and Staphylococci in chicken parts were inhibited by 3% polyphosphate solution, whereas the same concentration of polyphosphate failed to eliminate the growth of gram-negative microorganisms.

In a study conducted by Urbain (1983) to determine the effect of phosphate pretreatment, vacuum-packaging and radiation processing on fresh beef during storage at 5°C, it was discovered that high radiation doses (5 kGy) caused darkening of red meat (beef) and as the radiation dose increased, there was a corresponding increase in the amount of meat discoloration. Furthermore, pretreatment of the beef with tetrasodium pyrophosphate (TSPP) was found to protect beef color because discoloration of phosphate-treated, vacuum-packaged samples did not occur until an irradiation dose of 4 kGy was attained. In addition, a radiation dose of 1 kGy did not induce lipid oxidation in

beef samples stored at 5°C in the presence of tetrasodium pyrophosphate (TSPP)

Groesbeck (1983) also studied the microbiological characteristics of beef dipped in a 10% solution of tetrasodium pyrophosphate (TSPP) for 30 seconds and irradiated at a dose of 1 kGy. The samples were then stored at 5°C for 21 days in vacuum and 5 extra days in air. The results indicated that irradiated samples had higher counts of gram-positive microorganisms, while the unirradiated samples were dominated by gram-negative bacteria. However, no sporeforming rods were detected in any of the samples tested. It was further reported that Lactobacillus was found in all samples, and towards the end of storage, this group of organisms was found to predominate in the unirradiated samples.

Therefore, there may be chemical and microbiological advantages that would justify the addition of phosphates to meats that are to be irradiated.

Explanation of dissertation format

This study is divided in two parts, each being a complete paper already submitted (part 1) or to be submitted (part 11) to a Scientific journal.

The first paper covers the research carried out to determine the effect of low-dose (100 krad) gamma radiation and selected phosphates on the survival and growth of naturally occurring or inoculated bacteria in refrigerated (5°C), vacuum-packaged, ground pork.

The second paper covers the isolation and identification of bacterial cultures isolated from irradiated (100 krad) and nonirradiated, vacuum-packaged ground pork. The study was undertaken in order to contribute knowledge on the ability of meat bacteria to survive in pork subjected to low-dose irradiation under vacuum packaging conditions.

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EFFECT OF LOW-DOSE (1 kGy) GAMMA RADIATION
ON THE MICROFLORA OF VACUUM-PACKAGED GROUND PORK

by

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Running Title: Irradiation effects on ground pork bacteria

PART I. EFFECT OF LOW-DOSE (100 krad) GAMMA RADIATION
ON THE MICROFLORA OF VACUUM-PACKAGED GROUND PORK

ABSTRACT

Survival and growth of naturally occurring or inoculated bacteria was studied in refrigerated (5°C), vacuum-packaged, irradiated at 100 krad (1kGy) ground pork. Numbers of naturally occurring mesophiles, psychrotrophs, and anaerobes were reduced ($P < 0.01$) by irradiation, whereas lactic acid bacteria were unaffected. Partial bacterial recovery during subsequent storage at 5°C suggested sublethal bacterial injury due to irradiation. Irradiation prolonged shelf-life 2.5-3.5 days (30-44%) in uninoculated and 1.0-1.5 days in inoculated (10^5 CFU/g) meat. Added 0.4% sodium acid pyrophosphate (SAPP) contributed 2 additional days to inoculated, irradiated pork shelf-life, but had no effect on the naturally occurring microflora. Lipid oxidation did not increase significantly ($P > 0.05$) due to irradiation and was unaffected by phosphates.

INTRODUCTION

Ground pork is a highly perishable product that provides a very favorable medium for the growth of spoilage microorganisms. Treatment with low-dose gamma radiation has been proposed as a potential commercial process to extend the shelf-life of meats and contribute to consumer protection (Josephson, 1970; Froning, 1978; Niemand et al., 1981; Goresline, 1982). Ingram and Farkas (1977) summarized the relative radiation resistance of a wide variety of microorganisms, and Tawari and Maxcy (1971) had earlier stated that the common spoilage bacteria of refrigerated fresh meats were among the most radiation-sensitive microorganisms found in foods. Szczawinski (1984), studying the use of irradiation in combination with curing salts to control salmonella in meat products, reported that cells surviving irradiation at a dose of 3 kGy (300 krad) seemed to die at a faster rate at 0-2°C and showed delayed growth during storage at 8-10°C. The same author found that a 1-kGy (100 krad) dose strongly reduced the numbers of Salmonella spp. in ground meat. Dickson and Maxcy (1984), on the other hand, observed no inhibition of Escherichia coli or Moraxella-Acinetobacter inoculated on the surface of fresh meat previously irradiated at 15 kGy (1500 krad) and subsequently held at 5°C, indicating that no inhibitory

products were present in the irradiated meat and that the main radiation effects on bacteria in meat would be direct damage to the cells. A recent report on the microbiological effects of irradiation (100 krad) on fresh, vacuum-packaged pork loins, indicated that mesophilic, psychrotrophic, and anaerobic bacterial numbers were reduced by 100-krad gamma radiation processing (Mattison et al., 1986). Staphylococci numbers were similarly reduced.

Sivinski and Switzer (1983) also found that low-dose irradiation of pork at 30 krad sterilized trichinae larvae, and since all pork must be considered to potentially contain infective trichinae, the use of low-dose irradiation (30-100 krad) has been approved by the USDA (1985) to produce trichina-safe pork.

Despite the extensive research done on food irradiation, there is a lack of detailed information on irradiation processing of vacuum-packaged meats and its effect on the naturally occurring microflora. That has prompted the Food Safety Inspection Service (FSIS) of the USDA to ban the commercialization of irradiated, vacuum-packaged pork (Food Chemistry News, 1986). The main reason for such concern has been stated to be the possibility that hazardous microorganisms, particularly Clostridium botulinum, may grow in irradiated, vacuum-packaged meats. If the vacuum-packaged products were exposed to abuse

temperatures and other, more competitive, bacterial groups that would normally predominate and noticeably spoil the meat were destroyed by the irradiation process, such safety concerns would be justified. The work of Niemand et al. (1981) indicated that 2-kGy doses (200 krad) caused a drastic shift in the flora of prime beef cuts. As a result, there was an immediate predominance of lactic acid bacteria in radurized beef cuts that were not vacuum-packaged. Dickson and Maxcy (1985) demonstrated a similar, high survival rate by a lactic starter culture and destruction of staphylococci and coliforms in meat batches for fermented sausage production irradiated at doses as high as 500 krad.

Addition of food-grade phosphates has been reported to enhance the acceptability of irradiated fresh meats (Cohen et al., 1977). However, few reports are available on the effects of phosphates on the microflora of ground meats. Molins et al. (1986a) studied phosphate effects on the survival of bacteria in frozen beef patties. They found that none of the pure or blended phosphates tested at 0.4% contributed additional bacterial reductions when the patties were frozen, but some phosphates inhibited the growth of lactic acid microorganisms when the meat was held at room temperature (24-25°C) for 24 hr. The same authors showed that 0.5% sodium acid pyrophosphate, alone or combined with 100 ppm NaNO_2 , was inhibitory to mesophilic, psychrotrophic,

and anaerobic bacteria (including Clostridium sporogenes) in cooked, vacuum-packaged bratwurst (Molins et al., 1986b).

The objective of this work was to determine the potential use of low-dose irradiation (100 krad) to prolong the shelf-life of vacuum-packaged, ground pork. Additional objectives were to examine the relative survival of various spoilage bacterial groups in the meat after irradiation and to evaluate possible microbiological and chemical advantages of incorporating 0.4% selected phosphates to fresh, ground pork before vacuum-packaging and irradiation processing at 100 krad (1kGy).

MATERIALS AND METHODS

First Experiment

Fresh, refrigerated (2°C) pork trimmings (20% fat) were obtained from the Iowa State University Meat Laboratory and ground through a 1/8 in (31.8 cm) diameter plate in a Blonco SS250 grinder (S. Blondheim & Co., Inc., Oakland, CA). The meat was divided into 9.1 kg (20-lb) halves, and one-half was further subdivided into 227 g (0.5-lb) portions that were placed into 9 1/2 in L x 6 1/2 in W (24.13 cm L x 16.51 cm W) Curlon 892 (Curwod Inc., New London, WI) vacuum-packaging bags (O_2 permeability $< 1 \text{ mL}/645 \text{ cm}^2/24 \text{ hr}$ at 22.8°C and 0% RH). The meat was flattened by hand to minimize air pockets and to obtain an approximately even distribution throughout 3/4 of the bag length. The bags were then vacuum-sealed (1kPa) by a Multivac MG-2 (Sepp-Haggenmuller KG, West Germany) vacuum-packaging machine. After sealing, the meat in the bags was distributed throughout the sealed bags as before. These bags constituted the "uninoculated" treatment. The second half of the meat received an inoculum consisting of a mixture of nine psychrotrophic spoilage bacterial cultures isolated from ground pork and kept in the Iowa State Food Technology Department collection. The nine cultures had been

characterized as Pseudomonas spp. (5), Flavobacterium spp. (1), Acinetobacter spp. (1), Cardiobacterium spp. (1), and Vibrio spp. (1) by using the Minitek (BBL, Cockeysville, MD) bacterial identification system for non-fermenters. Before every replication of the experiment, the pure cultures were transferred from trypticase soy agar (TSA, BBL) slants into fresh, sterile brain heart infusion broth (BHI, BBL) tubes and incubated at 5°C for 10 days. To prepare the inoculum, 1 ml from each culture was aseptically pipetted into a dilution bottle containing 91 ml of sterile 0.1% peptone water. That protocol had previously been found to result in a suspension of ca. 10^7 cells/ml. The inoculum was added to the second half of the meat in the necessary volume to give approximately 10^5 cells/g of meat and thoroughly mixed in a Kitchen-Aid Model 4 mixer (The Hobart Manufacturing Co., Troy, OH). The meat was packaged as before and constituted the "inoculated" treatment. Eighteen bags of uninoculated ground pork and an equal number of inoculated meat bags were placed in a styrofoam-lined carton, layered with similar vacuum-sealed Curlon 892 bags containing crushed ice, sealed, labeled "to be irradiated" and placed in a 2°C cooler until shipment (1-2 hr). Previous trials had shown that the method effectively kept meat at low temperatures (<5°C) during transportation to the irradiation facility and back. A second carton was prepared by following the same

procedure but labeled "do not irradiate", in order to have a control set of meat that would travel and be subjected to the same temperature fluctuations as the irradiated meat. A third set of bags was not packaged into a carton but was placed in a display case at approximately 5°C. That set constituted an untraveled control, which was used to determine whether transportation conditions had adversely affected the microbiological quality of the meat. The cartons were shipped through an overnight delivery service to the irradiation facility operated by the Rocketdyne Division of Rockwell International Corp. in Canoga Park, CA. There, the cartons were opened and the bags from the carton labeled "to be irradiated" were irradiated at 100 krad (1.0 kGy), whereas the carton labeled "do not irradiate" had its lid removed and was allowed to remain at room temperature (24-25°C) for as long as the first set. After the appropriate set was irradiated, all bags were returned to the Iowa State University Food Technology Laboratory. The trip was completed within 42 hr. of preparation of the samples with the exception of one replication of the experiment (out of a total of 4 replications) for which unexpected transportation problems arose. The temperature of the meat ranged from 3 to 12°C upon arrival in the irradiation facility. During the time necessary for irradiation, the meat being irradiated as well as the

nonirradiated controls reached 18-21°C, while returning meat temperatures ranged from 2 to 5°C when received at the Iowa State University Food Technology Laboratory. Upon arrival, the cartons were opened, and all samples were placed in a display case at about 5°C.

A set of samples from the controls that did not travel was examined for bacterial numbers on the day the meat was prepared (day 0), and the data were considered to represent day 0 data for all samples. Thereafter, a set of samples from each treatment was taken from the display case and analyzed on days 3 (the day samples returned to ISU), 6, and 9. Uninoculated samples were also examined on day 12. A spoilage level of 10^7 aerobic mesophilic or psychrotrophic colony-forming units (CFU) was defined, based on the work of Kraft and Ayres (1952). Trypticase soy agar (TSA, BBL) was used to enumerate aerobic mesophiles (30°C/48 hr) and psychrotrophs (5°C/10 days). Lactic acid producing bacterial numbers were determined with lactobacilli specific medium (LBS, BBL; 30°C/72 hr), while anaerobic microorganisms were enumerated by using TSA (BBL) supplemented with 1% soluble starch (Fisher Scientific, Fair Lawn, NJ). Anaerobic incubation (30°C/48 hr) conditions were obtained by using Anaerobic Gas Pak Systems (BBL). On sampling days, bags of meat were aseptically opened and 30-g samples were macerated for 1 minute with 270 ml of 0.1%

sterile peptone water in a Stomacher 400 Lab Blender (Tekmar Co., Cincinnati, OH). Serial dilutions were prepared following standard procedures. Chemical measures included pH, determined with a Radiometer 28 pH meter (Radiometer, Copenhagen, Denmark) equipped with an Orion 9163 probe (Orion Research, Cambridge, MA). Thiobarbituric acid (TBA) values in the meat were determined by the distillation method of Tarladgis et al. (1960) in 3 replications of experiment 1 and in 2 replications of experiment 2. Plate count data were transformed to logarithms, and all data were analyzed by using a Statistical Analysis System (SAS, 1986) computer program with a general linear model procedure. Comparison of means was based on Duncan's multiple range test. The experiment was replicated four times.

Second Experiment

The procedure for preparing the samples followed the same outline described for the first experiment, except that additional treatments were included. Meat batches containing 0.4% (w/w) sodium acid pyrophosphate (SAPP) (Stauffer Chemical Co., Westport, CT) or Brifisol 414 phosphate blend (BK Ladenburg, Cresskill, NJ) were prepared by adding the appropriate volumes of a 10% (w/v), filter-sterilized (22- Millipore filters, Millipore Corp.,

Bedford, MA) solution of one phosphate to 1000-g portions of ground pork. Phosphate selection was based on the apparent effectiveness of SAPP and Brifisol 414 in relation to bacterial growth inhibition in cooked, vacuum packaged bratwurst (Molins et al., 1986a). After the phosphate was blended in with a Kitchen-Aid Model 4 mixer (The Hobart Manufacturing Co., Troy, OH) for 1 min, the meat was weighed, placed in bags, vacuum-sealed, randomly layered with the meat corresponding to experiment 1 in insulated cartons, shipped, irradiated (or not irradiated), returned to ISU, and examined as before. Two replications of the second experiment were performed.

Dosimetry and irradiation

Irradiation of the samples was carried out at the Rockwell International Gamma Irradiation facility, a panoramic, dry storage irradiator, containing approximately 30 kCi of cobalt-60 and 650 kCi of cesium-137 isotopes. Eighteen cobalt-60 pencils (totaling approximately 17.5 kCi of source strength), held in a rectangular, 61 x 61 cm, aluminum rack were utilized for irradiating the samples. Vacuum-packaged ground pork samples similar to those to be used in the study were held in the form of a 3 x 3 x 4 array, in a specially fabricated, 46 x 46 x 6 cm sample

holder made from two 0.5-cm thick plexiglas sheets separated by wooden spacers. Prior to the four replicate irradiations, a 17-hour dosimetry calibration test was performed using test samples, with 81 radiochromic film dosimeters (Far West Technology, Goleta, CA) placed at selected locations within the sample holder, and between the outer plastic surfaces of the samples, to determine dose rate and dose uniformity. The dosimeters were made of hexahydroxyethyl pararosanine nitrile in polyamide (nylon), designed for gamma dosimetry in the range of 0.1-3.0 Mrad (1-30 kGy). The dosimeters used for the test were from Rocketdyne Lot 4, and their dose calibration was traceable to the U.S. National Bureau of Standards.

In recognition of the fact that the dosimeters were not sensitive in the dose region of interest (100 krad), test samples and dosimeters were exposed to a total dose in the range 407-526 krad (4.07 - 5.26 kGy) during the calibration test. The optical density changes of the exposed dosimeters were then measured with a Model 92 film reader (Far West Technology, Goleta, CA).

During the four replicate irradiations, all relevant experimental parameters, including the distance between the source rack and sample holder (76 cm), the source strength, and the sample geometry were maintained the same as in the calibration test so as to achieve the identical dose rate.

The exposure time (approximately 3.5 hr) for each test was established precisely from the calibration test data, accounting for the time-dependent decay of the cobalt-60. The sample holder was rotated 180° during irradiation to further enhance dose uniformity. This procedure, combined with appropriate modifications to the calibration test data to account for the rotation, resulted in the samples receiving irradiation doses in the range 92-99 krad (0.92-0.99 kGy). The procedure also resulted in the exterior surfaces of the two outermost samples in the holder, located along the central horizontal line between the source rack and the sample holder receiving the desired maximum dose of 100 krad (1.0 kGy). The standard deviation (one-sigma) for these data was estimated to be $\pm 4\%$.

RESULTS AND DISCUSSION

Possible temperature fluctuations during transportation of the samples to the irradiation facility and back, as well as meat temperature increase during exposure to room temperature (24-25°C) at the irradiation site, significantly ($P < 0.05$) affected bacterial numbers in the meat. Nonirradiated controls that traveled alongside the samples to be irradiated, whether inoculated or not, had consistently higher ($P < 0.05$) numbers of aerobic mesophilic or psychrotrophic microorganisms, as well as higher lactic acid bacteria and anaerobic counts, than did controls that did not travel. Although the data corresponding to microbial growth in controls that remained in a display case (5°C) in our laboratory at all times are not presented, this result justified the inclusion of a full set of controls that traveled but were not irradiated. Comparisons between irradiated and nonirradiated treatments in this report, therefore, were limited to the two sets of samples that traveled to the irradiation facility and back.

Figures 1 through 4 present the growth curves corresponding to mesophilic, psychrotrophic, anaerobic and lactic acid bacteria, respectively, throughout the experimental period. Irradiation significantly ($P < 0.01$) reduced the number of mesophilic bacteria (Fig. 1).

However, the large difference between the number of mesophiles present in irradiated or in nonirradiated samples, whether inoculated or not, 24 hr after irradiation (day 3), remained significant ($P < 0.05$) but became considerably smaller after 6 days at 5°C. This result suggested that irradiation at 100 krad (1kGy) injured a sizable portion of the mesophilic flora of vacuum-packaged ground pork, but was not always lethal. A second possibility is that some irradiated bacteria were temporarily hindered by the dialyzable inhibitor reported by Bruns and Maxcy (1978) to occur in trypticase soy agar. Such compound was found to inhibit the recovery of a radiation-resistant Moraxella sp. Anaerobic bacteria (Fig. 3) behaved in a pattern similar to mesophiles, whereas radiation-induced lethality was greatest among the psychrotrophic microorganisms (Fig. 2).

When 10^7 CFU/g of meat is taken to be the level beyond which the meat would be considered spoiled (horizontal line in Figs. 1-4), uninoculated, vacuum-packaged ground pork treated with 100 krad of gamma radiation had 3.5 more days of useful life in terms of psychrotrophic total counts (from 8 to 11.5 days, Fig. 2). In relation to anaerobic bacterial numbers, meat shelf-life would have been extended from 8.5 to 11 days (Fig. 3). Shelf-life extension would have been shortest when aerobic mesophilic bacteria are considered (1

day), but this group of microorganisms would not be critical in determining the shelf-life of refrigerated, vacuum-packaged ground pork unless the packaging film had a high oxygen permeability or there were air leaks into the package and the meat temperature rose above 10°C. The importance of preventing bacterial contamination during meat handling was highlighted by the comparatively shorter time to reach 10^7 CFU/g observed in inoculated meat. When the initial number of mesophiles or anaerobes was $>10^5$ (Figs. 1 and 3, respectively), irradiation at 100 krad contributed only 1-1.5 additional days to shelf-life (from 4.5 to 6 days), and 3 days in relation to psychrotrophic bacteria (Fig. 2).

In contrast to the effects of 100 krad of gamma radiation on mesophilic, psychrotrophic, and anaerobic microorganisms, lactic acid producing bacteria appeared to be unaffected (Fig. 4). Initial numbers of those organisms were low (10 CFU/g) and were similar in inoculated and uninoculated samples because no such bacteria were included in the inoculum. The growth curves corresponding to irradiated samples indicated that the irradiation process delayed the growth of the lactics by approximately 1 log cycle for 6 days, but no differences ($P>0.05$) were evident between the populations of lactic acid bacteria in irradiated meat samples and in nonirradiated controls thereafter. After 9 days at 5°C, however, the uninoculated,

nonirradiated pork contained higher ($P < 0.05$) lactic acid bacterial numbers than its uninoculated, irradiated counterpart (Fig. 4). The lactics in nonirradiated controls reached the 10^7 CFU/g level on day 12, while an extrapolation of the growth curve of lactic acid bacteria in irradiated, uninoculated controls (Fig. 4) indicated that such a level would also have been reached in those samples in approximately 15 days. That observation suggested that vacuum-packaged pork irradiated at 100 krad followed the same pattern of spoilage observed in nonirradiated meat, but had a considerably longer shelf-life. Nevertheless, although lactic acid bacteria in uninoculated, irradiated samples did not reach 10^7 CFU/g during the 12-day storage period at 5°C , the meat presented obvious signs of spoilage (purge, slime, discoloration, and off-odors) after 12 days, when other nonlactic microbial numbers approached the previously defined 10^7 CFU/g spoilage level.

The results of the second experiment are not presented graphically for uninoculated ground pork. In general, the addition of 0.4% phosphates to the meat did not result ($P > 0.05$) in long-term additional inhibition of any bacterial group studied. Only Brifisol 414 caused a temporary, significant ($P < 0.05$) drop in the number of naturally occurring anaerobic microorganisms upon irradiation. That effect continued for as long as 6 days of storage at 5°C but

disappeared thereafter. Anaerobic growth was also inhibited for 3 days in samples that contained SAPP or Brifisol 414 and were not irradiated.

Data for inoculated samples obtained in the second experiment (Figs. 5-8) indicated that phosphate addition without irradiation did not extend the shelf-life of the meat. However, unlike samples without phosphates or treated with Brifisol 414, the initial inhibition ($P < 0.05$) of mesophilic, psychrotrophic, and anaerobic bacterial growth attributable to irradiation was maintained significantly ($P < 0.05$) longer in pork meat that contained 0.4% SAPP. As with uninoculated meat, irradiation or irradiation plus SAPP addition delayed lactic acid bacteria by ca. 1 log cycle throughout the experimental period (Fig. 8). The overall contribution of 0.4% SAPP plus irradiation was a shelf-life extension of approximately 2 days over that of samples irradiated only (ca. 8 vs. 6 d, respectively). Together with the extension of meat shelf-life contributed by irradiation alone, SAPP provided a total extra shelf-life of more than 3 days (75%) over that of inoculated vacuum-packaged, untreated ground pork.

No significant ($P > 0.05$) pH differences between treatments were detected, although mean pH values in samples treated with phosphates were 0.1-0.2 units above untreated ones, whether irradiated or not.

Thiobarbituric acid values (TBA) did not increase significantly ($P>0.05$) as a result of irradiation (Table 1) but were usually higher in irradiated samples after 9 days of storage at 5°C. Except for inoculated, irradiated meat samples, TBA values never reached the generally accepted human threshold value of 1.0. This agreed with similar values reported by Mattison et al. (1986) in vacuum-packaged pork loins irradiated at 100 krad. Phosphate addition did not result in significant ($P>0.05$) reductions in TBA values of irradiated pork.

CONCLUSIONS

Exposure of vacuum-packaged ground pork to a dose of 100 krad (1kGy) of gamma radiation caused an immediate reduction ($P < 0.01$) in naturally occurring mesophilic and anaerobic colony forming units (CFU). Irradiation seemed to have injured rather than destroyed many of those organisms, a large proportion of which were able to recover after 6 days at 5°C. Radiation-induced lethality was greatest among psychrotrophic bacterial populations, whereas lactic acid producing microorganisms were unaffected by the 100-krad radiation dose.

Microbiological spoilage of irradiated (100 krad) vacuum-packaged ground pork followed the same pattern observed in nonirradiated meat, but spoilage was delayed by radiation processing. Shelf-life of irradiated, uninoculated, vacuum-packaged ground pork was extended 2.5-3.5 days (30-44%) in relation to nonirradiated meat and on the basis of time to reach 10^7 CFU/g total anaerobic or psychrotrophic bacterial counts, respectively. In samples inoculated with ca. 10^5 CFU/g, spoilage was faster than in uninoculated ones and irradiation prolonged shelf-life only 1.0-1.5 days (22-33%).

The addition of 0.4% pure sodium acid pyrophosphate (SAPP) or of a phosphate blend (Brifisol 414) did not

adversely affect ($P>0.05$) naturally occurring bacterial numbers in irradiated or nonirradiated, vacuum-packaged ground pork. In inoculated meat, 0.4% SAPP contributed 2 days to shelf-life in addition to those attributable to irradiation alone, for a total shelf-life extension of 75%. Injury to mesophilic, psychrotrophic, or anaerobic bacteria from radiation processing was made permanent by the presence of 0.4% SAPP in inoculated samples, while the growth of lactics was delayed no different in uninoculated or inoculated, irradiated pork with or without SAPP.

Thiobarbituric acid values (TBA) in ground pork were not significantly ($P>0.05$) affected by irradiation, although irradiated meat samples had consistently higher TBA values than nonirradiated pork. Mean TBA values only exceeded 1.0 in inoculated, irradiated meat after 9 days at 5°C. Phosphate addition did not affect ($P>0.05$) TBA values in irradiated ground pork.

Table 1. Mean TBA values in irradiated (100 krad) and nonirradiated vacuum-packaged ground pork (mg malonaldehyde/1000 ml)

<u>Treatment</u>	<u>Days at 5°C</u>	
	<u>3</u>	<u>9</u>
1. <u>Uninoculated meat</u>		
a. Nonirradiated	0.27	0.95
b. Irradiated	0.22	0.57
c. Nonirradiated + 0.4% SAPP ^a	0.36	0.49
d. Irradiated + 0.4% SAPP ^a	0.33	0.67
e. Nonirradiated + 0.4% Brifisol 414 ^a	0.22	0.51
f. Irradiated + 0.4% Brifisol 414 ^a	0.52	0.47
2. <u>Inoculated meat</u>		
a. Nonirradiated	0.22	0.87
b. Irradiated	0.47	1.34
c. Nonirradiated + 0.4% SAPP ^a	0.17	0.43
d. Irradiated + 0.4% SAPP ^a	0.24	0.52
e. Nonirradiated + 0.4% Brifisol 414 ^a	0.14	0.55
f. Irradiated + 0.4% Brifisol 414 ^a	0.24	0.76

^aMean of 2 replications.
Standard error: 0.04.

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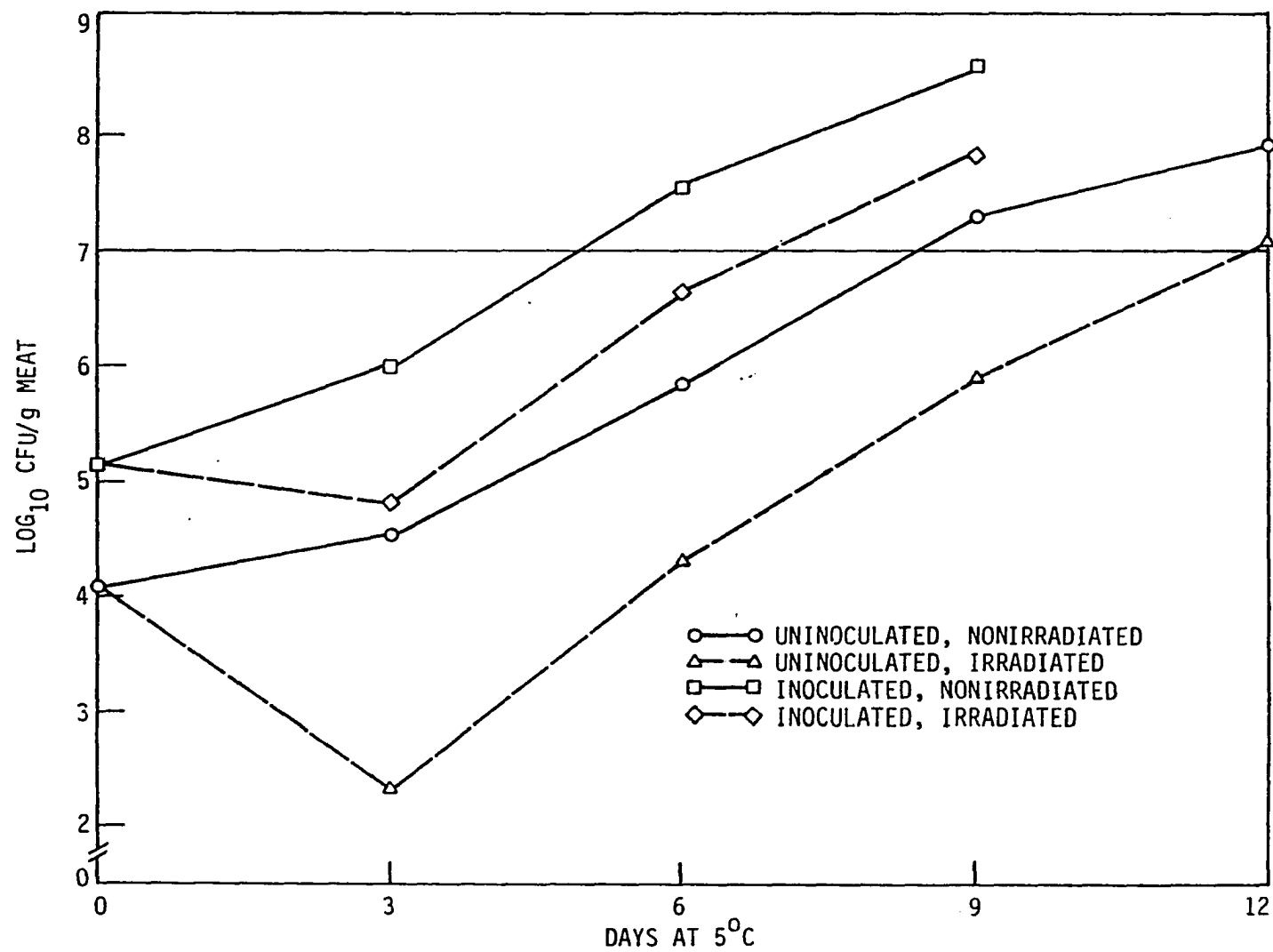
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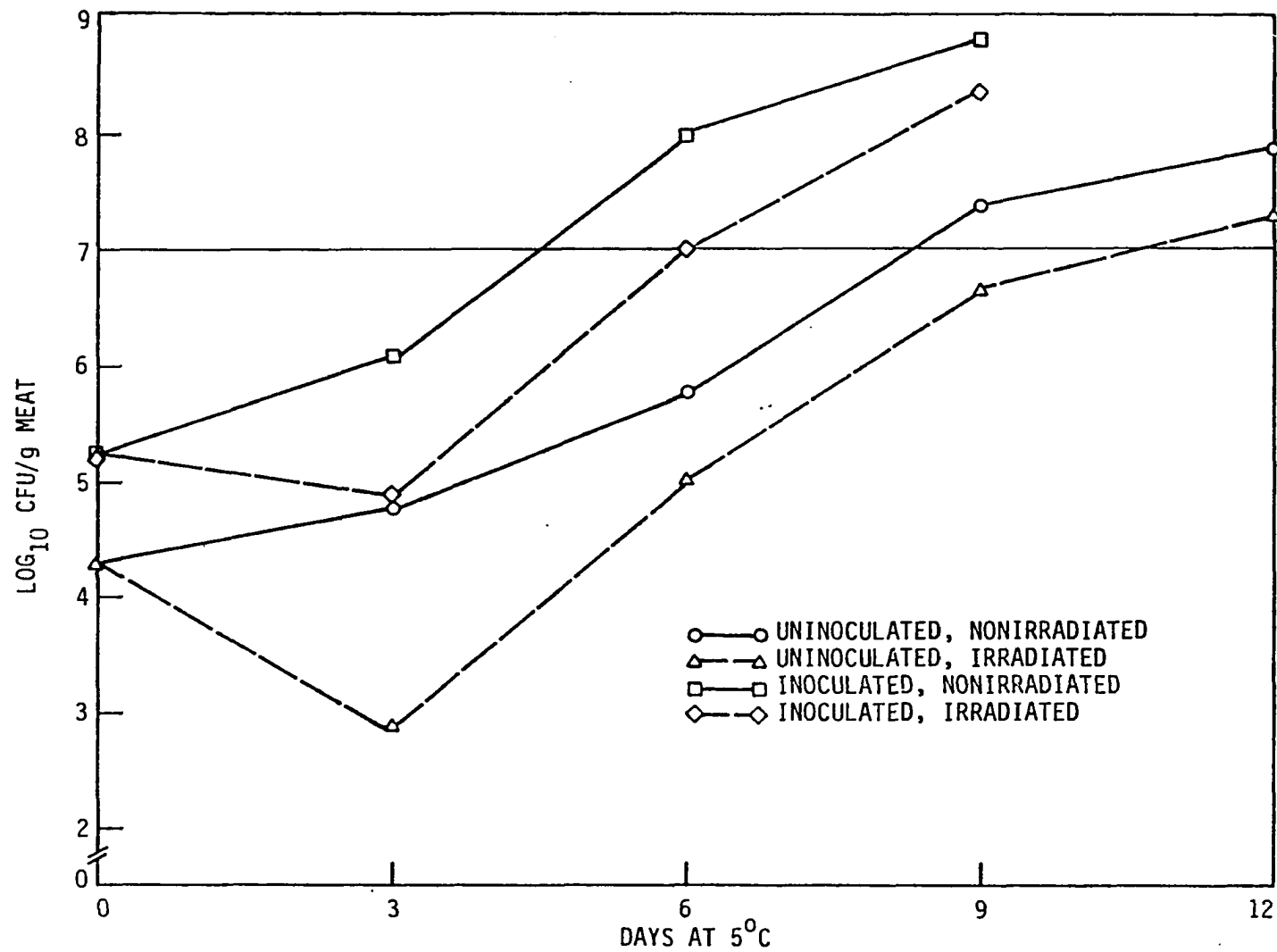
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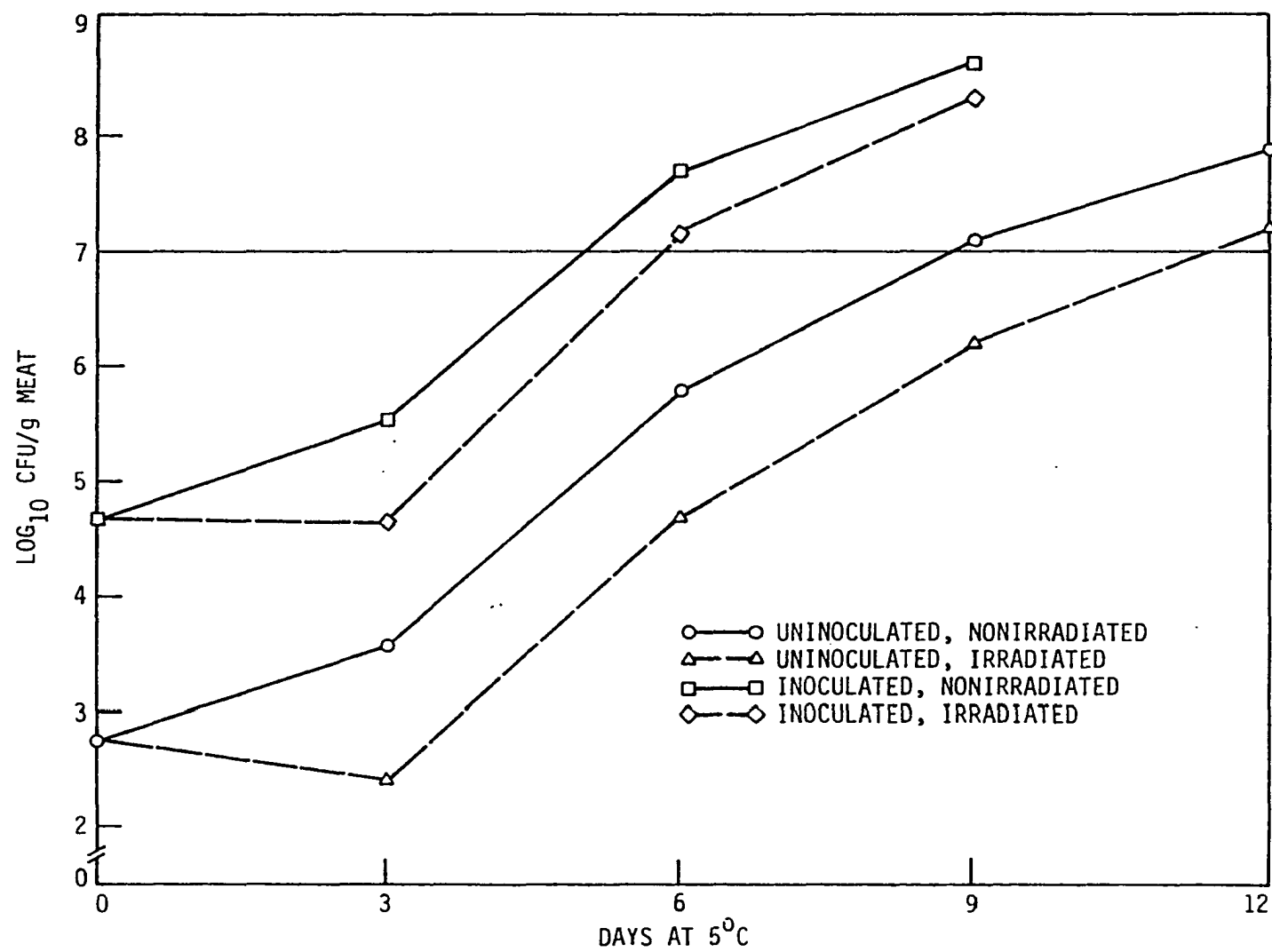
**Figure 1. Growth of mesophilic bacteria in vacuum-packaged,
irradiated ground pork**



**Figure 2. Growth of psychrotrophic bacteria in vacuum-packaged,
irradiated ground pork**



**Figure 3. Growth of anaerobic bacteria in vacuum-packaged,
irradiated ground pork**



**Figure 4. Growth of lactic acid bacteria in vacuum-packaged,
irradiated ground pork**

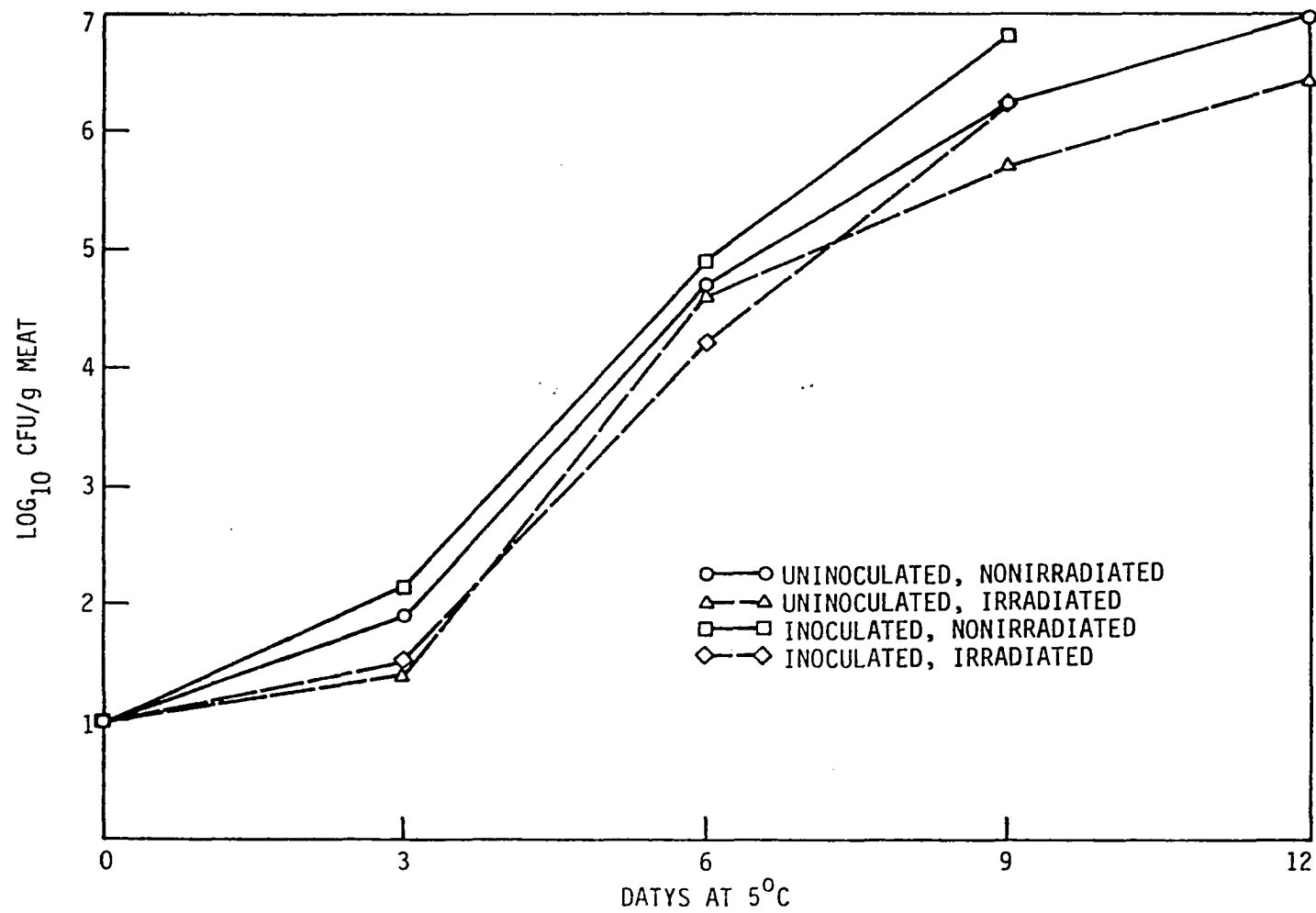


Figure 5. Effect of 0.4% phosphates on survival and growth of mesophiles in vacuum-packaged, irradiated ground pork

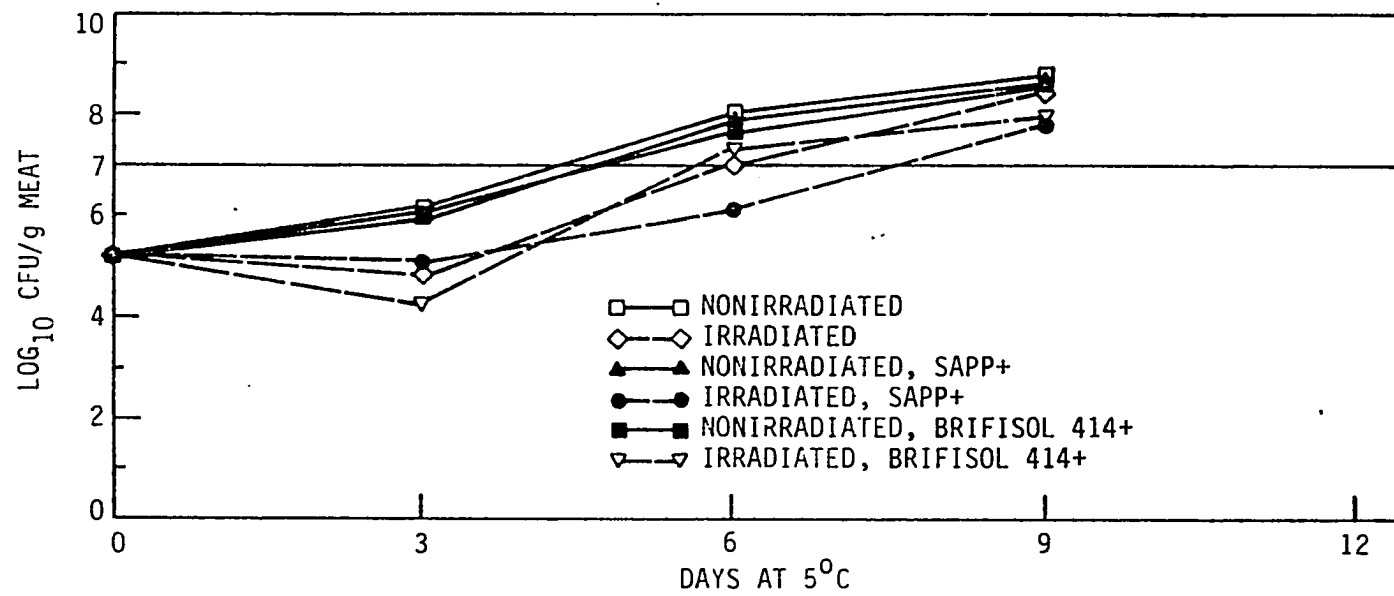


Figure 6. Effect of 0.4% phosphates on survival and growth of psychrotrophs in vacuum-packaged, irradiated ground pork

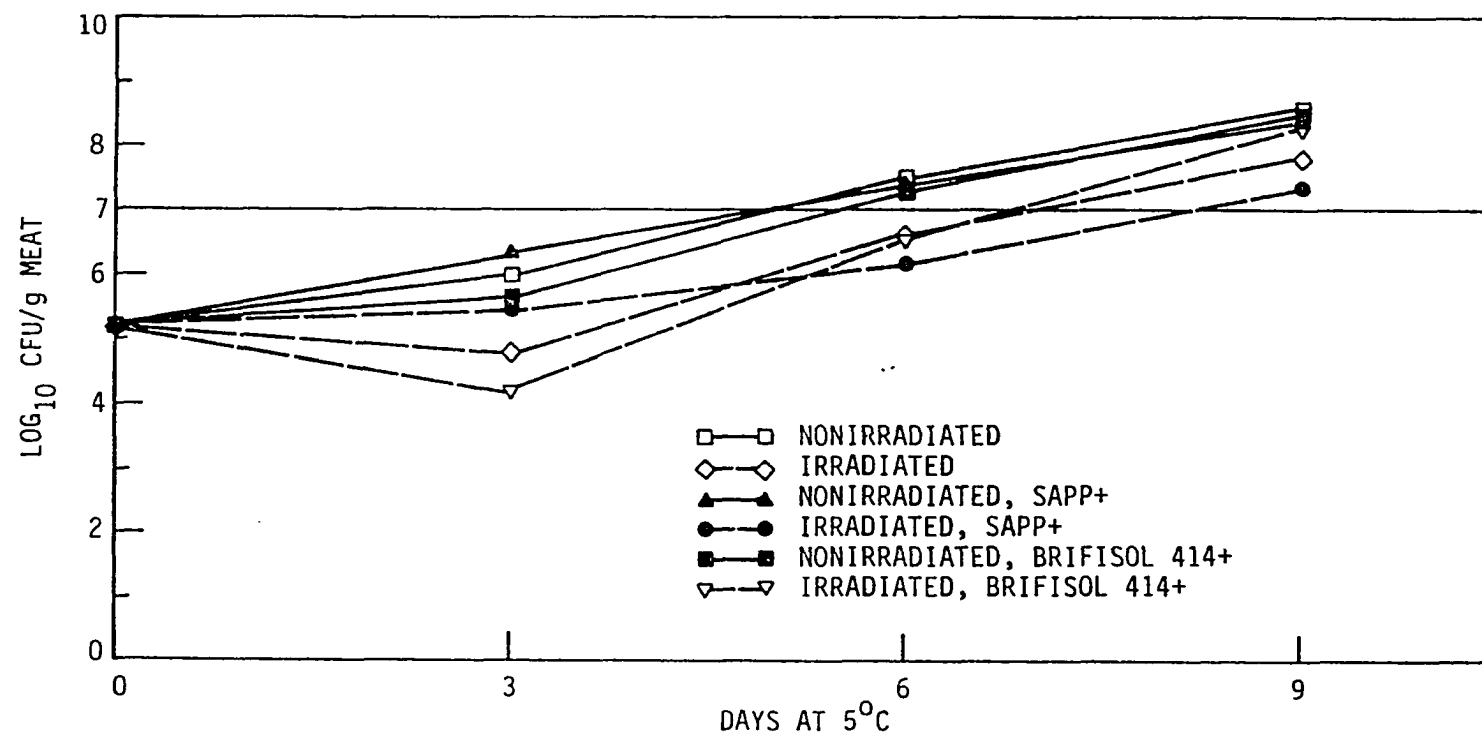


Figure 7. Effect of 0.4% phosphates on survival and growth of anaerobes in vacuum-packaged, irradiated ground pork

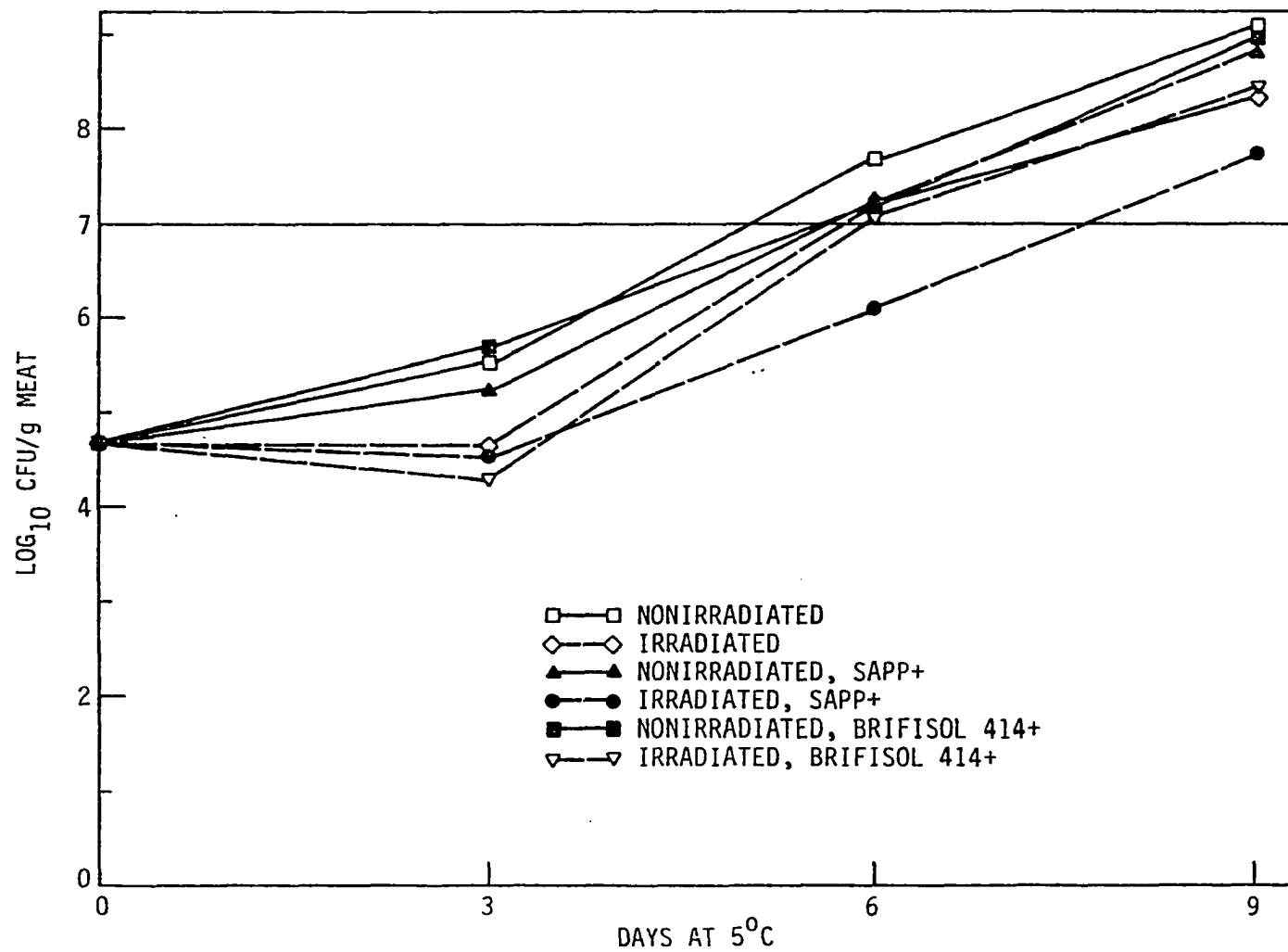
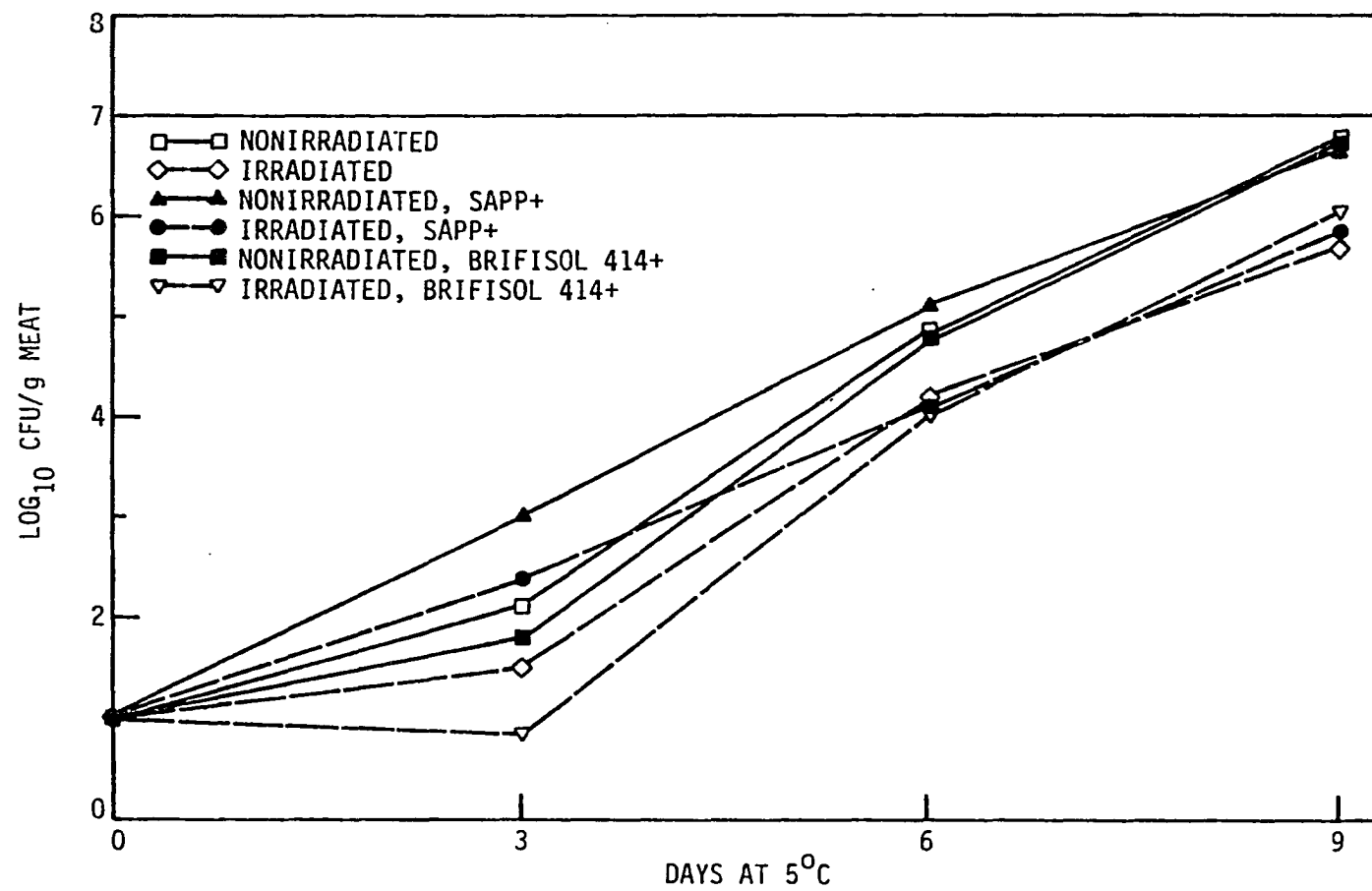


Figure 8. Effect of 0.4% phosphates on survival and growth of lactic acid bacteria in vacuum-packaged, irradiated ground pork



IDENTIFICATION OF MICROBIAL ISOLATES FROM
VACUUM-PACKAGED GROUND PORK IRRADIATED
AT 100 KRAD (1KGY)

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PART II. IDENTIFICATION OF MICROBIAL ISOLATES FROM
VACUUM-PACKAGED GROUND PORK IRRADIATED AT
100 KRAD (1KGY)

ABSTRACT

Bacterial cultures from irradiated (100 krad, 1 kGy) and nonirradiated, vacuum-packaged ground pork held at 5°C were isolated and characterized over a 12-day storage period. The initial flora of the meat was composed mostly of Pseudomonas sp. and Enterobacter sp. Although the microflora of nonirradiated samples gradually shifted from Gram-negative to Gram-positive microorganisms, 76% of the isolates were characterized as Gram-negative at the onset of spoilage (9 days at 5°C). In contrast, the irradiated ground pork microflora was mainly Gram-positive (66%) shortly after irradiation and increased to 97% after 9 days at 5°C. A total of 720 isolates were identified to genus.

INTRODUCTION

Fresh meats contain a broad spectrum of microorganisms arising from contamination during animal slaughter, dressing and fabrication operations. The bacterial genera more often associated with spoilage of fresh, refrigerated red meats stored under conditions of oxygen availability are Pseudomonas, Moraxella-acinetobacter, Flavobacterium, Alcaligenes and members of the Enterobacteriaceae, all Gram-negative organisms, although Gram-positive genera such as Corynebacterium and other non-spore formers may be of importance (Newsome et al., 1984; Simard et al., 1984; Kraft, 1986a). Upon reducing oxygen availability through vacuum packaging and continued meat tissue respiration, the strictly aerobic spoilage flora of fresh, refrigerated meats has been shown to be inhibited (Sutherland et al., 1975; Seideman et al., 1976a,b; Lee et al., 1984, Kraft, 1986b). After refrigerated storage periods of 7-21 days and depending on such variables as meat pH, holding temperature and oxygen permeability of the packaging film, Gram-positive, facultatively anaerobic, lactic acid producing bacteria such as Lactobacillus spp., Micrococcus spp. and Streptococcus spp. become predominant (Sutherland et al., 1975; Seideman et al., 1976a; Christopher et al., 1979; Egan and Shay, 1982; Hitchener et al., 1982). Their growth

eventually results in meat spoilage through discoloration and souring (Hodges et al., 1974; Taylor and Shaw, 1977; Kraft, 1986a). However, other bacterial genera have also been shown to survive and/or grow in vacuum-packaged, refrigerated meats, including Brochothrix thermosphacta (Roth and Clark, 1975; Grau et al., 1985) and, most important, members of the Enterobacteriaceae.

Irradiation of fresh meats and poultry is considered a potentially useful technology to increase product shelf-life (Niemand et al., 1981, 1983; Bok and Holzapfel, 1984; Mattison et al., 1986) and to reduce or eliminate potential pathogenic microorganisms (Kampelmacher, 1983; Thayer et al., 1986). It has also been demonstrated that lactic acid bacteria are, in general, relatively resistant to gamma radiation injury compared to pathogens such as Staphylococcus aureus and some Enterobacteriaceae (Dickson and Maxcy, 1985). However, the known reduction of spoilage bacteria effected in meats by low and medium gamma radiation doses (<100 krad and 100-1000 krad, respectively) has prompted concerns about the possibility of pathogenic microorganisms being able to survive, grow and pose a threat to public health in irradiated, mishandled, vacuum-packaged meats. That concern was evidenced by the Food Safety Inspection Service (FSIS) banning of vacuum-packaged pork irradiation (Anonymous, 1986).

The present work was undertaken to determine the identity of bacterial cultures isolated from fresh, vacuum-packaged pork irradiated at 100 krad (1 kGy) and stored at 5°C until spoilage, compared to that of isolates from similar, nonirradiated ground pork.

MATERIALS AND METHODS

Fresh ground pork obtained from the Iowa State Meat Laboratory was prepared, vacuum-packaged, shipped overnight to be irradiated at 100 krad (1 kGy) at the facility operated by the Rocketdyne Division of Rockwell International Corporation, Canoga Park, CA, shipped back to Iowa State University and stored in a display case for up to 12 days at approximately 5°C. The procedures followed in meat preparation, shipment, dosimetry and irradiation were previously reported by Ehioba et al. (1987). A set of control samples traveled alongside those to be irradiated but were not irradiated.

Meat samples were taken from the display case on the day of preparation (day 0), on day 3 (the day samples returned to ISU) and on days 6, 9 and 12 of storage at 5°C for microbiological analysis. Therefore, day 0 samples were all nonirradiated controls. Portions weighing 30 g were placed in sterile plastic bags and blended for 1 min with 270 ml sterile 0.1% peptone water by using a Stomacher 400 Lab Blender (Tekmar, Cincinnati, OH). Serial dilutions were prepared in the standard way. Although trypticase soy agar (TSA, BBL) was used to enumerate mesophilic and psychrotrophic bacteria (Ehioba et al., 1987), separate, duplicate plates of the appropriate dilutions were plated

with all-purpose tween agar (APT, BBL) and inoculated at 30°C for 48 hr for bacterial culture isolation. That was done to ensure the recovery of as many bacterial genera as possible, in view of the known failure of many lactic acid producing bacteria, particularly lactobacilli, to grow on widely used, general purpose media (Rogosa, 1961). In addition, APT agar was deemed to be a better medium than TSA for isolation of cultures from irradiated meats because Bruns and Maxcy (1978) had found that TSA contained a dialyzable factor that inhibited irradiated bacteria.

Twenty colonies were taken from each of the duplicate plates following a diagonal line drawn on the outside of the plate bottom to assure randomness. This resulted in 40 isolates per treatment, sampling day and experimental replication. Well isolated colonies were picked with a sterile needle from countable APT plates corresponding to irradiated or nonirradiated samples, streaked onto APT plates and allowed to grow for 48 hr at 30°C. The isolates were then transferred to APT slants, incubated at 30°C for 24 hr and stored at 15°C for future identification.

Culture isolation and identification procedures were replicated twice, using the samples of replications No. 3 and 4 of the study by Ehioba et al. (1987). Consequently, 80 isolates per treatment and sampling period were characterized, for a total of 720 isolates. Cultures

isolated from nonirradiated ground pork corresponding to day 0 (sample preparation day) were taken to be representative of the microflora of the meat before irradiation.

Before proceeding with the identification, the isolates were transferred twice to fresh APT slants, incubated 24 hr at 30°C and grouped on the basis of Gram reaction and cell morphology. Gram-positive cultures were characterized following the scheme of Harrison et al. (1981) and the criteria of Bergey's Manual of Systematic Bacteriology (1986). Gram-negative isolates were tested for oxidase reaction and growth on McConkey's medium. On that basis, Gram-negative isolates were identified using the Minitek miniaturized differentiation systems for nonfermenters and miscellaneous Gram-negative bacilli, or for Enterobacteriaceae (Enterobacteriaceae II) (BBL Microbiology Systems, Cockeysville, MD).

RESULTS AND DISCUSSION

Table 1 presents the overall grouping of the isolates by Gram reaction, treatment (nonirradiated or irradiated meat) and storage time at 5°C. The microflora of the meat on the day of preparation was composed of mostly Pseudomonas spp. and Enterobacter spp., which together constituted 63% of the bacterial population examined. Gram-negative isolates totalled 96% of the initial flora (Table 2). A gradual shift in the microflora of nonirradiated samples took place at 5°C. Upon return of the meat to ISU (day 3), Gram-negative isolates had decreased to 67 (84%), while Gram-positive cultures had increased from only 3 on day 0 to 13 (16%). After 9 days at 5°C, when nonirradiated, vacuum-packaged ground pork had already surpassed the 10^7 colony forming units (CFU)/g defined by Ehioba et al. (1987) as the experimental spoilage criterion, 61 out of 80 isolates were gram-negative (76%). In contrast, Gram-positive isolates dominated the flora of vacuum-packaged, ground pork irradiated at 100 krad (1 kGy) immediately after irradiation (day 3). At that time, total mesophilic and psychrotrophic bacterial counts in irradiated meat decreased by almost 2 log cycles (from 4.3 to 2.9, and from 4.1 to 2.2, respectively) compared to those in controls (Ehioba et al., 1987). That might explain the relatively large proportion

(25%) of yeasts isolated from the meat on the first sampling period following irradiation (day 3, Table 1).

Upon prolonged storage at 5°C (9 and 12 days), Lactobacillus spp. and coryneforms predominated in irradiated samples, whereas Gram-negative bacteria were rarely found. In contrast, nonirradiated samples had spoiled by day 9 at 5°C as reported earlier (Ehioba et al., 1987) and reached the spoilage level of 10^7 CFU/g with 96% Gram-negative microorganisms as part of the spoilage microflora. This result suggested that, from the standpoint of product safety in relation to Enterobacteriaceae, vacuum-packaged ground pork irradiated at 100 krad should not raise more concerns than its nonirradiated counterpart. A small percent of Gram-negative isolates were considered to be Yersinia spp. Although Yersinia were present in low numbers after 12 days at 5°C, their survival in vacuum-packaged, irradiated (100 krad) ground pork, would justify additional research. Yersinia spp. were also detected in nonirradiated samples but, unlike the irradiated product, the nonirradiated meat was spoiled (i.e., had surpassed 10^7 CFU/g) at day 9 at 5°C (Ehioba et al., 1987). Irradiated samples did not reach the predefined spoilage level until after 11 days at 5°C.

The results of this study agreed with those of Niemand et al. (1983) in that irradiated ground pork contained 90%

or more Gram-positive bacteria at the end of the 12-day refrigerated storage period (Table 2). However, such predominance of lactic acid bacteria and other Gram-positive isolates, in our study, was evident shortly after irradiation.

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Mention of any company or product does not constitute endorsement.

Table 1. Microflora of irradiated (100 krad) and nonirradiated, vacuum-packaged ground pork stored at 5°C.

	<u>Treatment, sampling day and number of isolates^a</u>									
	<u>Nonirradiated meat</u>					<u>Irradiated meat</u>				
	<u>Days at 5°C</u>					<u>Days at 5°C</u>				
	0	3	6	9	12	0	3	6	9	12
I. Gram-negative Isolates										
<u>Pseudomonas</u>	36,14	33,10	18,17	25,8	5,3	7,9	--	--	--	--
<u>Enterobacter</u>	0,18	0,16	0,4	0,5	16,10	0,2	--	--	--	--
<u>Serratia</u>	--	--	--	--	5,0	0,2	--	--	--	--
CDC Group VE-1	--	--	--	3,5	--	--	--	--	--	--
<u>Flavobacterium</u>	--	--	--	2,0	--	1,0	--	--	--	--
<u>Acinetobacter</u>	1,1	0,2	0,4	1,2	2,0	--	--	--	--	0,3
<u>Yersinia</u>	--	--	--	--	7,0	--	--	--	--	2,0
<u>Chromobacterium</u>	--	0,1	--	1,2	--	--	--	--	--	--
<u>Alcaligenes</u>	0,5	1,1	0,6	--	--	--	--	--	--	--
Unidentified ^b	2,0	0,3	3,1	5,2	5,1	3,3	--	--	1,1	0,4
Subtotal	39,38	34,33	21,32	37,24	40,14	11,16	--	--	1,1	2,7
Total Gram-negative ^a	77	67	53	61	54	27	0	0	2	9

II. Gram-positive
Isolates

<u>Lactobacillus</u>	--	--	15,5	--	--	0,2	5,17	--	14,19
<u>Coryneform</u>	--	5,	3,2	1,3	--	2,0	2,6	39,26	0,11
<u>Micrococcus</u>	--	0,1	--	--	--	4,3	0,9	0,4	--
<u>Streptococcus</u>	--	--	--	0,2	0,3	1,3	0,7	--	0,1
Yeasts	--	--	0,2	0,2	--	6,14	--	0,9	--
Unidentified ^c	1,2	1,6	--	6,5	0,23	16,2	33,1	--	24,2
<hr/>									
Subtotal	1,2	6,7	18,9	7,12	0,26	29,24	40,40	39,39	38,33
Total Gram- positive ^d	3	13	27	19	26	53	80	78	71

^aNumber of isolates from replications 3 and 4 of the study by Ehioba *et al.* (1987).

^bCultures with biochemical profiles outside the Minitex data base.

^cGram-positive cultures lost due to mold contamination.

^dTotal number of isolates giving - or + Gram reaction out of a total of 80 isolates per sampling period.

Table 2. Percent Gram-negative and Gram-positive isolates composing the microflora of vacuum-packaged, irradiated (100 krad) and nonirradiated ground pork stored at 5°C

	<u>Nonirradiated meat</u>					<u>Irradiated meat</u>				
	<u>Days at 5°C</u>					<u>Days at 5°C^a</u>				
	<u>0</u>	<u>3</u>	<u>6</u>	<u>9</u>	<u>12</u>	<u>0</u>	<u>3</u>	<u>6</u>	<u>9</u>	<u>12</u>
Gram negative isolates										
Total No. ^b	77	67	53	61	54	77	27	0	2	9
%	96	84	66	76	68	96	34	0	3	11
Gram-positive isolates										
Total No. ^b	3	13	27	19	26	3	53	80	78	71
%	4	16	34	24	32	4	66	100	97	89

^aSampling following irradiation (100 krad).

^bNumber of isolates of a total of 80 per sampling period.

SUMMARY

The effectiveness of low-dose gamma irradiation (100 krad), alone, and low-dose gamma irradiation plus selected phosphates in prolonging the shelf-life of vacuum-packaged, ground pork were demonstrated in this study. In the first experiment, the use of low-dose gamma radiation resulted in immediate reduction of naturally occurring mesophiles and anaerobes. However, after 6 days storage at 5°C, most of the radiation injured microorganisms recovered and continued to grow. The psychrotrophs were most radiation sensitive of all the microbial groups studied, while the lactic acid bacteria were most radiation resistant. Uninoculated, vacuum-packaged ground pork was found to have an additional shelf-life of 3.5 days in relation to psychrotrophic bacterial growth after exposure to 100 krad of gamma radiation. When the shelf-life of the vacuum-packaged ground pork was considered in terms of anaerobic microorganisms, the shelf-life was found to have been extended by 2.5 days. Extension of shelf-life in relation to aerobic mesophilic bacteria was shortest (1 day), but the importance of this group of microorganisms in determining the shelf-life extension of refrigerated, vacuum-packaged ground pork is not very critical, unless, of course, the packaging material would allow oxygen to penetrate and the

temperature of the meat were above 10°C. In inoculated (ca. 10⁵ CFU/g) vacuum-packaged meat samples, the shelf-life of the product was extended by only about 1.0-1.5 days. The significance of maintaining clean surroundings during meat handling to discourage bacterial contamination of the product is supported by the findings in this study. It took the inoculated, irradiated meat samples far less time to reach 10⁷ CFU/g (level beyond which meat is considered spoiled) than the uninoculated, irradiated samples. In contrast to the effects of 100 krad gamma radiation on mesophilic, psychrotrophic and anaerobic bacteria, the effect of 100 krad gamma radiation on lactic acid bacteria was much less pronounced. Though the initial numbers of lactic acid bacteria were low in both the inoculated and uninoculated meat samples, low-dose gamma radiation was found to cause a delayed growth response of the lactic acid bacteria by approximately 1 log cycle for 6 days. However, there were no differences between the lactic acid bacterial populations in irradiated and nonirradiated vacuum-packaged ground pork. In the uninoculated, nonirradiated meat samples, it was found that the populations of lactic acid bacteria were higher after 9 days storage at 5°C than in uninoculated, irradiated meat samples. While the lactic acid bacteria in the nonirradiated samples reached 10⁷ CFU/g in 12 days, it took 15 days for those microorganisms to

reach 10^7 CFU/g in uninoculated, irradiated meat samples.

Apart from the temporary drop in numbers of naturally occurring microorganisms attributable to Brifisol, addition of 0.4% phosphates to vacuum-packaged ground pork did not result in prolonged additional inhibition of any of the bacterial groups studied, irradiated or nonirradiated. However, 0.4% sodium acid pyrophosphate (SAPP) was found to prolong the shelf-life of inoculated meat by 2 days in addition to those contributed by irradiation alone. The combined effect of low-dose (100 krad) gamma radiation plus 0.4% SAPP resulted in permanent injury to mesophilic, psychrotrophic and anaerobic microorganisms, whereas radiation alone resulted in temporary injury such that the organisms were able to resume growth after a few days storage at 5°C. The inclusion or noninclusion of 0.4% SAPP did not have any additional effect on the delayed growth pattern of lactic acid bacteria in inoculated or uninoculated, irradiated pork.

Results obtained from the chemical measurements indicate that there were no significant ($P>0.05$) differences in pH values between treatments. However, it was found that phosphate-treated meat samples had mean pH values 0.1-0.2 units higher than untreated, irradiated or unirradiated samples. Though the thiobarbituric acid (TBA) values were shown to be higher in irradiated samples after 9 days at

5°C, it was discovered that irradiation did not have a significant ($P>0.05$) effect on overall TBA values. Only in inoculated meat samples did TBA values exceed the threshold value of 1. Furthermore, the addition of phosphate did not induce a significant ($P>0.05$) reduction in TBA values of irradiated pork.

Results from the second part of this investigation showed that the microorganisms inherent in preirradiated, vacuum-packaged ground pork differed to a great extent from those present in post-irradiated meat samples. It was found that nonirradiated ground pork contained mostly gram-negative microorganisms of which Pseudomonas was most prevalent. This group of microorganisms was found to predominate in four of five sampling times. However, there was an observable decrease in the number of gram-negatives in irradiated meat samples as storage time approached 12 days.

Low-dose irradiation (100 krad) was found to eliminate most of the gram-negative Pseudomonas spp. and a few gram-positive microorganisms. On further storage however, Lactobacillus spp. were found to predominate in the irradiated vacuum-packaged meat samples. A noticeable microflora shift from gram-negative microorganisms in nonirradiated samples to

mostly gram-positive microorganisms in 100 krad gamma radiation-treated meat samples was noted.

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